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ON THE ARTIFICIAL EXTRAPAUSE OF THE VENTRICLE OF THE FROG'S HEART

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Dastre (1) and Langendorff (2) were the first to show that sometimes after applying an artificial stimulus to the auricles of the frog's heart, a prolonged ventricular pause arises, which is not initiated by an extrasystole of the ventricle. Engelmann (3) was in position to corroborate this experiment and to elucidate it. He pointed out that the experiment succeeds only when the stimulus is given to the auricles at the commencement of the ventricular systole, after which an extrasystole of the auricles will ensue. After this the excitation wave proceeds to the ventricle and reaches it before the close of the refractory stage, so that no ventricular systole follows. Only after the compensatory pause which succeeds the extrasystole of the auricles do the auricles and the ventricle resume their normal rhythm. This experiment, however, seldom succeeds. It is instanced in figure 1. At *T* the first upward deflection of the signal the auricles were given an induction shock[1] at the commencement of the ventricular systole. After the auricular extrasystole evoked by this shock the excitation reached the ventricle during the refractory stage, so that no systole of this chamber arose.

Not before the end of the compensatory pause of the auricles did an auricular systole arise again, followed by a ventricular systole. I have now been more successful in this experiment, by lengthening the dura-

[1] In all figures the closing of the primary circuit was indicated by a downward deflection of the signal. At the opening of the primary circuit an upward deflection of the signal was effected. In figures 1, 2, 3, 4 and 6 the closing stimuli were shut off and consequently they did not reach the heart.

tion of the refractory stage of the ventricle. Then the excitation wave after the artificial extrasystole of the auricles will with greater certainty reach the ventricle still in the refractory stage. This lengthening of the refractory stage of the ventricle may be effected in different ways. First of all we know ever since Langendorff wrote, that the duration of the postcompensatory systole has increased. I now found that during the postcompensatory systole also the duration of the refractory stage has increased. It may be expected, therefore, that the experiment succeeds better during a postcompensatory systole. This may be seen from figure 1, in which the auricles received a fresh stimulus during the postcompensatory systole at the second upward deflection of the signal, and hereafter followed another extrapause of the ventricle, which was not preceded by a premature ventricular systole.

At *P* the third upward deflection of the signal the auricles were again stimulated at the commencement of a ventricular systole. After the evoked extrasystoles of the auricles the excitation wave reached the ven-

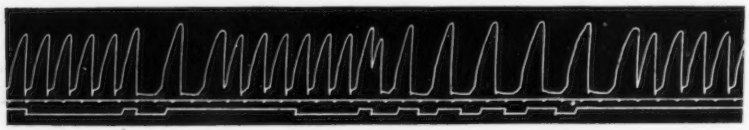


Fig. 1

tricle after the refractory stage, so that a premature ventricular systole ensued. When, however, at the next upward deflection of the signal the stimulus is repeated at the commencement of the postcompensatory systole, the excitation wave after the extra-auricular systole thus evoked, readily reaches the ventricle during the refractory stage. Now an extrapause of the ventricle follows. In this way it is easy to repeat the experiment during every following ventricular systole, which is broadened every time. At last it is even unnecessary to stimulate the auricles at the commencement of the ventricular systole, the last stimulus being given about the middle of the ventricular systole without diminishing the success of the experiment. This, indeed, is easily understood, if we look more carefully at the ventricular systoles of this artificial halved ventricular rhythm. We then observe that after the compensatory pause the postcompensatory systole is broader than the preceding ventricular systoles, and that every succeeding systole surpasses its predecessor in broadness. We see then that the contractility of the ventricular

muscle increases after every lengthened ventricular pause. This restoration of the ventricular muscle in the artificial halved rhythm involves an increase in duration of the refractory stage from systole to systole. This is why ultimately the stimulus can be administered to the auricles later in the ventricular period, without interfering with the success of the experiment. After the last stimulus the ventricle resumes again the normal rhythm.[2] In the second place we can lengthen the refractory stage of the ventricle by poisons, namely digitalis, veratrin, antiarin or barium chloride and, by doing so, ensure success of our experiment. The curves of figure 2 refer to a frog's heart that had been poisoned with barium chloride. At every upward deflection of the signal the auricles receive an opening induction shock at the commencement of a ventricular systole. Every time there appears an extrasystole of the auricles and every time after this the excitation reaches the ventricle during the refractory stage, so that extrapauses of the ventricle orig-

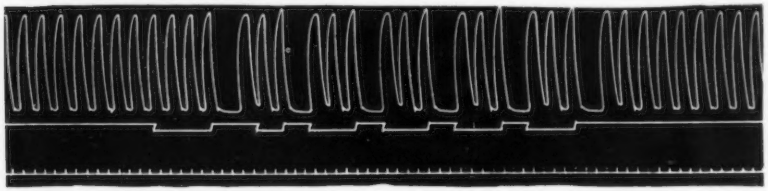


Fig. 2

inate, which are not preceded by premature ventricular systoles.[3] I have now detected that artificial extrapauses of the ventricle may be evoked in the frog's heart in quite another manner. Whereas in the method described above, the prolongation of the refractory stage of the ventricle was the decisive factor, the following method is based on a principle unknown as yet in the physiology of the heart. When we place the stimulating electrode in the auriculo-ventricular groove, we can evoke under certain circumstances (prolonged refractory stage of the ventricle), by the administration of an extrastimulus toward the

[2] After poisoning with veratrin, digitalis, antiarin or barium-chloride, the halved rhythm of the ventricle can persist after one or more extra-pauses of the ventricle, without stimulating the heart any more. This also can occur after bleeding the non-poisoned frog's heart. (See fig. 5.)

[3] In a later stage of this intoxication the ventricle maintains its pulsation in the halved rhythm after such an artificial extrapause.

close of the diastole of the ventricle, an extrapause of the ventricle, which is not preceded by an extrasystole of this chamber.

In our experiments described above we had to give the extra stimulus at the beginning of the systole to obtain the desired result. When the stimulus was given a little later a premature ventricular systole succeeded the extrasystole of the auricles.

It is obvious, then, that when a stimulus at the end of the diastole of the ventricle produces the same effect, it cannot be explained in the same way. We shall therefore illustrate the latter experiment by some curves. In figure 3 we see a reproduction of the suspension curves of a frog's heart after veratrin poisoning. (The heart was left in situ and the circulation of the blood was left intact; some drops of 1 per cent sol. acetas veratrini had been injected into the dorsal lymph sac about

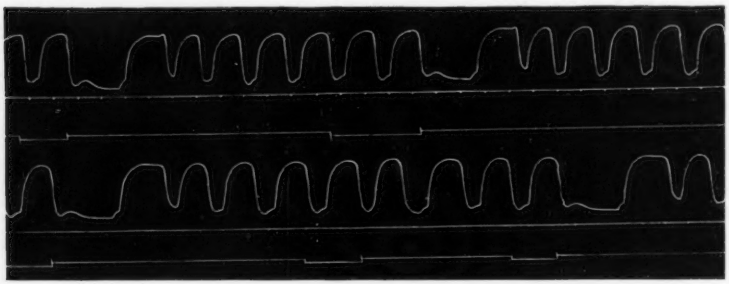


Fig. 3

10 minutes before.) At the first upward deflection of the signal an opening induction shock was given. After this we see an auricular systole represented in the suspension curve, which is not followed by a systole of the ventricle. Just as in the experiments described above, an extrapause of the ventricle follows after this auricular systole. At the next upward deflection the same experiment was repeated in the upper row of curves with the same result. Now when measuring the curve we find that the auricular systole, which appeared a short time after each of the two stimuli, follows after the commencement of the preceding auricular systole with an interval of a sinus period. We therefore applied the extra stimulus in the auriculo-ventricular groove a short time before the commencement of a normal periodic auricular systole. At that moment the ventricle was apparently still refractory, as there did not appear an extrasystole of the ventricle. The auricles,

however, respond to the stimulus. The excitation wave now traverses the auricles from the auriculo-ventricular boundary in the direction of the sinus venosus.

But simultaneously, the periodic sinus impulse traverses the auricles in an opposite direction. The two excitations meet and rebound. At that moment the auricular systole is accomplished under the influence of two excitation waves, passing through the auricles in opposite direction. The excitation waves clash against each other and are annihilated. Now we understand that the auricular systole succeeding the extra stimulus, originates partly under the influence of the periodic sinus impulse and partly from the extra stimulus.[4]

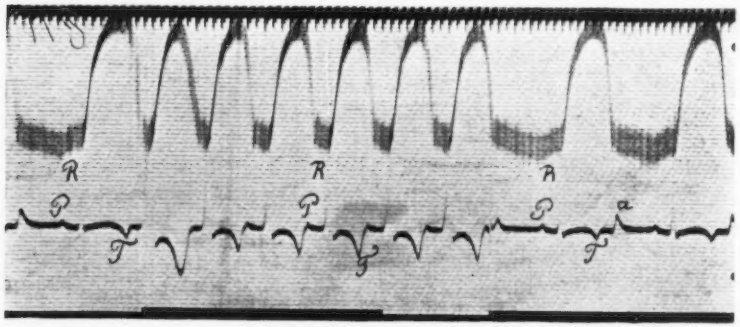


Fig. 4

It is also clear that this auricular systole cannot in this case be followed by a ventricular systole. In the lower curves, registered a little later, this experiment is repeated with the same result at the first and the third upward deflection of the signal. At the second upward deflection of the signal the stimulus is given a little later, so that then an extrasystole of the ventricle appears. In figure 4 are illustrated the suspension curves and the electrograms of a frog's heart after antiarin poisoning. Initially the ventricle pulsated in halved rhythm, which at the first upward deflection of the signal was changed into the normal rhythm of twice the velocity. At the second upward deflection of the signal another induction shock is administered in the auriculo-ventric-

[4] It goes without saying that it depends on the moment, at which the extra-stimulus is administered to which impulse the greater part of the auricular systole owes its origin. So, for instance, in figure 6 the two auricular systoles will arise for the greater part from the extra stimulus.

ular groove.[5] We see from the string curve that this stimulus is administered a short time after the P-deflection. At this moment the ventricle is apparently still refractory, so that an extrasystole of the ventricle is not evoked.[6] The auricles, however, do respond to the stimulus, so that these are now traversed at the same time by an excitation wave in a retrograde direction. This excitation wave, which traverses the auricles after the extra stimulus, encounters in the auricles the periodic sinus impulse, which was already on its way from the opposite side at the moment when the extra stimulus was given. Both excitation waves are then annihilated, so that no premature ventricular systole can follow and an extrapause of the ventricle manifests itself. Thereafter the normal ventricular rhythm is transposed into the halved rhythm.[7] It is beyond doubt that in this case the greater part of the auricular systole is owing to the periodic sinus impulse, because this impulse was already traversing the auricles at the moment when the extra stimulus was being administered. We have seen heretofore that at the moment when the extra stimulus in the auriculo-ventricular groove is administered, the ventricle must be refractory. To ensure the success of this experiment it will be well to lengthen the refractory stage of the ventricle.

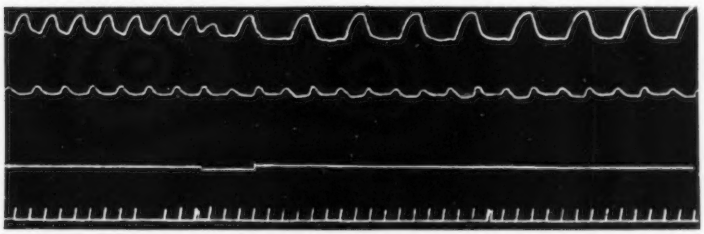
In the two preceding experiments we have effected this lengthening by veratrin or by antiarin poisoning. We can now avail ourselves also of the fact that the refractory stage of the ventricle is lengthened by the postcompensatory systole. This is instanced in figure 5. It repre-

[5] The moment at which the extra stimulus is applied is marked by the signal and may also be seen from the string curve, which shows a small gap owing to a short swerving of the string.

[6] In the string curve we see directly after the stimulus, a small triangular deflection, which tells us that after all an extremely small part of the ventricle is contracted. We are safe to conclude that the sinus impulse can not rebound on this extremely small partial contraction, since, indeed, in the frog's heart the auricles are interconnected with the ventricle all along the auriculo-ventricular groove (auriculo-ventricular funnel). Similarly we see in figure 3 a slight difference in the magnitude of the deflections of the suspension curve, after the four stimuli which initiate the extra-pauses of the ventricle. Very likely also here an extremely small portion of the ventricle has been made to contract once or twice.

[7] I need not enlarge upon these transpositions of rhythm and the changes they involve for the ventricle electrograms. They were discussed by me in *Koninklyke Academie van Wetenschappen te Amsterdam Proceedings* XX, 696; (1917), 271 and 502. *Archives Néerl. de Physiologie*, iii (1918), 7 and 90. *Pflüger's Archiv*, Bd. 173, S. F. S. 1918.

sents the suspension curves of the auricles (lower curves) and of the ventricle (upper curves) of a frog's heart after bleeding. The stimulating electrode is applied in the auriculo-ventricular groove. At the downward deflection of the signal a closing shock is administered.[8] This gives rise to an extrasystole of the ventricle, which is followed by a compensatory pause. During the postcompensatory systole an opening shock is applied. Although this shock was administered at the commencement of an auricular systole just as the previous shock, the result is quite different. The refractory stage of the postcompensatory systole, namely, is lengthened, so that at the moment when the stimulus is applied the ventricle is still refractory and consequently presents no extrasystole. The auricles, however, do respond to the stimulus at the auriculo-ventricular boundary, so that consequently an excitation wave traverses the auricles in retrograde direction. This



[Fig. 5

excitation wave encounters in the auricles the periodic sinus impulse, so that both excitation waves are annihilated and no premature ventricular systole can follow. After the extrapause of the ventricle, thus originating, the following systole of the ventricle is extended and broadened. Now because this systole engenders a prolonged refractory stage of the ventricle, the ventricle is caught in the halved rhythm.[9] It is evident that the previously described experiments succeed only when the extra stimulus affects the auriculo-ventricular groove at a special moment.

If that moment coincides with the moment at which the periodic sinus impulse enters the auricles, the experiment will succeed. Success

[8] In this figure the closing induction shocks are not shut off and are announced by a downward deflection of the signal.

[9] These transpositions of rhythm in the bled frog's heart will be discussed in the following communication.

will even be achieved when the extra stimulus is applied somewhat later or earlier. In figure 4, e.g., at the second upward deflection of the signal, it was applied shortly after the P-deflection, therefore shortly after the periodic impulse had entered the auricles from the sinus venosus. In figure 6 the experiment succeeded twice through extra stimuli which were applied shortly before the P-deflection in the auriculo-ventricular groove. At the first upward deflection of the signal the extra stimulus was applied on the peak of the negative T-deflection, i.e., still before the P-deflection would be registered.[10] The excitation wave then traverses the auricles in a retrograde direction and encounters the periodic sinus impulse in the vicinity of the sinus venosus. The P-deflection, which otherwise would have revealed itself directly after the close of the T-deflection, does not appear now. The auricular systole is somewhat premature in this case and may

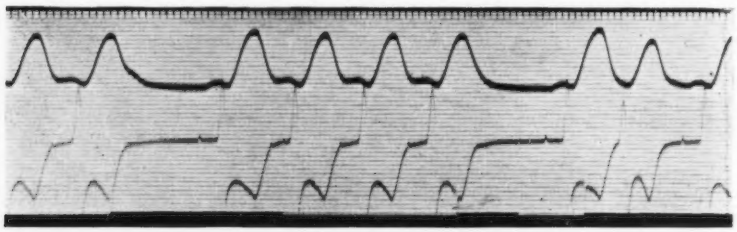


Fig. 6

still just be seen in the suspension curve in the last part of the ventricular diastole. It is obvious that this auricular systole is chiefly owing to the extra stimulus. At the second upward deflection of the signal the stimulus was applied a little before the peak of the T-deflection. The result is similar to that with the previous stimulus, viz., an extrapause of the ventricle.

If the extra stimulus is applied much later or earlier than the moment at which the sinus impulse enters the auricles, no extrapause of the ventricle will follow. If later the extra stimulus will affect the ventricle after the refractory stage and an extrasystole of the ventricle will ensue, followed by a compensatory pause. This is illustrated in figure 3, in the lower curves at the second upward deflection of the signal.

[10] In the electrogram we see the P-deflections appear directly after the close of the T-deflections.

Conversely, when the stimulus is given much earlier, an extrasystole of the auricles is originated, after which a systole of the ventricle follows at a prolonged a-v interval. An instance of this case is given in figure 6 at the third upward deflection of the signal.

At the first and the second upward deflection of the signal the extra stimulus was applied at the peak of the T-deflection or a short time before it, which resulted in an extrapause of the ventricle. At the third upward deflection of the signal, however, the extra stimulus was applied much earlier, viz., rather more than $\frac{1}{2}$ second before the peak of the T-deflection. It appears that the auricles respond already to the stimulus and present a complete extrasystole, but this retrograde excitation is not stayed in its course by the periodic sinus impulse in the auricles but probably in the sinus venosus. After this auricular extrasystole the excitation wave proceeds to the ventricle and induces it to contract.

Success of the latter experiment depends upon various conditions:

1. The extra stimulus is to affect the auricles after the refractory stage of these chambers.

2. After the artificial extrasystole the excitation wave is to reach the ventricle after its refractory stage.

Finally I wish to advert to the necessity of amplifying Engelmann's interpretation of the constant duration of the compensatory pause in connection with the present investigation. According to Engelmann the reason why, instead of the extrasystole a normal periodic ventricular systole has fallen out, is because the periodic sinus impulse reached the ventricle during the refractory stage of the extrasystole. The present research induces me to add that in some cases the periodic ventricular systole falls out because after the extra stimulus the excitation which proceeds also in retrograde direction, clashes upon the periodic sinus impulse, so that both excitations are annihilated.

When we thus amplify the interpretation of the duration of the compensatory pause, a fact becomes clear to me that had been known to me long since, namely that when an extra stimulus is given to the ventricle, we see in some of the experiments, during the extrasystole a P-deflection expressed in the electrograms, in others we do not. If the P-deflection is absent it is obvious that the periodic sinus impulse has not traversed the whole auricle, but has been stayed in its course by the excitation proceeding in retrograde direction, evoked by the extra stimulus.

SUMMARY

If we evoke an extrasystole of the auricles in the beginning of a ventricular systole, in some cases this extrasystole is not followed by a systole of the ventricle. This experiment succeeds only when, after the extrasystole of the auricles, the excitation wave reaches the ventricle before the close of the refractory stage. It was shown, that this experiment succeeds with more certainty if we lengthen the refractory phase by poisoning the frog's heart with veratrin, digitalis, antiarin or barium chloride. In the second place we know that the duration of a postcompensatory systole has increased and also the refractory phase of a postcompensatory systole has increased. Therefore does the experiment also succeed with more certainty if we evoke an extrasystole of the auricles in the beginning of a postcompensatory systole. In the third place we can evoke a prolonged pause of the ventricles in quite another way. We prolong the refractory phase of the ventricle and apply an induction shock in the auriculo-ventricular groove *toward the close of the diastole* and before the close of the refractory phase of the ventricle. Therefore an extrasystole of the ventricle does not appear but the auricles respond to the stimulus. The excitation wave traverses the auricles from the auriculo-ventricular groove in the direction of the sinus venosus. But simultaneously the periodic sinus impulse traverses the auricles in an opposite direction. The two excitation waves clash against each other and are annihilated. In this case the auricular systole is accomplished under the influence of two excitation waves passing through these two chambers in opposite directions. It is clear, that this auricular systole cannot be followed by a ventricular systole.

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RESEARCHES ON THE RHYTHM AND METABOLISM OF THE BLED FROG'S HEART

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The following facts were among others stated by me in the pharmaco-physiological investigation I made on frogs' hearts, after I had poisoned them with veratrine, digitalis, antiarine or barium chloride (1).

1. The duration of the refractory stage of the ventricle muscle increases after the administration of each of these poisons, and so does likewise the a-v interval; at last the contractility of the ventricle muscle decreases.

2. As soon as the relative duration of the refractory stage

$$\left(\frac{\text{duration of the total refract. stage}}{\text{duration of a sinus period}}\right)$$

surpasses the value 1, suddenly or gradually the normal ventricle rhythm changes into the halved one.

a. The sudden halving of the ventricle rhythm comes about in the following manner: The duration of the refractory stage of the ventricle has increased during the normal rhythm of the ventricle for the reason that the ventricle muscle was not yet entirely restored at the beginning of every ventricular systole. What was still wanting to this restoration, was called by me the *residual refractory stage.*

The periodical refractory stage was added to it by every systole, so that the total refractory stage consists of two components. If now the relative duration of the refractory stage has become longer than 1, the next following ventricular systole falls away, and a protracted ventricular pause is the consequence. This protracted pause influences the two components in an opposite sense.

The ventricle muscle restores itself better, so that the residual refractory stage decreases. But after a protracted pause the next following systole of the ventricle is considerably enlarged, consequently the duration of the periodical refractory stage of the ventricle increases.

If now this increase of the duration of the periodical refractory stage surpasses the decrease of the residual refractory stage, then suddenly halving of the ventricle rhythm sets in.

b. The gradual transition to the halved ventricle rhythm however, takes place when the decrease of the residual refractory stage surpasses the increase of the periodical refractory stage. For, if this takes place, the normal ventricle rhythm continues after a protracted pause, till by accumulation the duration of the residual refractory stage causes again the falling away of a ventricular systole, and the normal ventricle rhythm is resumed again. So groups of ventricular systoles come into existence, which become gradually smaller and smaller, till in the end the halved ventricle rhythm is reached in this way.

3. Spontaneous alternations between the halved ventricle rhythm and the normal one occur frequently. The cause of these alternations lies in the fact that during the halved ventricle rhythm the katabolic index of the ventricle

$$\left(\frac{\text{duration of the total refract. stage of the ventricle}}{\text{duration of a ventricular period}}\right)$$

decreases again by restoration, till it has become less than one-half. Then the normal ventricle rhythm sets in again. In this twice as rapid ventricle rhythm[1] the katabolic index of the ventricle increases again under the influence of the small pauses of the ventricle and consequently the halved rhythm of the ventricle sets in again. So these alternations can repeat themselves several times.

4. By extra stimulation of the ventricle the halved ventricle rhythm can be artificially converted into the normal twice-as-rapid rhythm by the intercalation of one little ventricular systole. This proves that during the halved rhythm of the ventricle the sinus impulses that are not answered by the ventricle, did really reach this chamber of the heart, but rebounded on the yet refractory ventricle muscle.

The normal ventricle rhythm can likewise be converted into the halved one by extra stimulation. The enlarged postcompensatory systole fixed then the ventricle in the halved rhythm. I attributed these and many other results, not mentioned here, to the fact that an important factor of the action of the heart, viz., the refractory stage had been modified under the influence of the employed poisons. Its

[1] During the normal ventricle rhythm the katabolic index of the ventricle is equal to the relative duration of the refractory stage.

duration increased by veratrine, digitalis, antiarine and barium chloride. These poisons had no further possible mysterious actions for the results mentioned above.

SPONTANEOUS TRANSITION OF THE NORMAL INTO THE HALVED VENTRICLE RHYTHM OF THE NOT POISONED FROG'S HEART

The following observations made with regard to not poisoned frog's hearts afforded an unmistakable affirmation of the before-mentioned facts. *The before-mentioned sudden and gradual transition into the halved ventricle rhythm occurs likewise in the not poisoned frog's heart, the spontaneous alternations between the halved rhythm of the ventricle and the normal one can also be stated.* In figure 1[2] we give a reproduc-

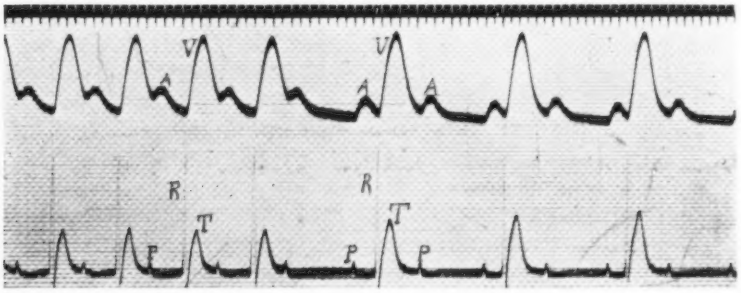

Fig. 1

tion of the suspension curves and the electrograms of a frog's heart (rana esculenta). More than an hour after the suspension this heart shows constantly repeated alternations between the normal ventricle rhythm and the halved one. I succeeded in photographing such a spontaneous alternation under simultaneous registration of the action currents.

This reproduction shows a great number of important details and affords a formal confirmation likewise for not-poisoned frogs' hearts, of the theoretical explanations communicated by me in former essays. In the figure we see suddenly appear the halved ventricle rhythm after four normal ventricular systoles. Three of these are still regis-

[2] Constantly one electrode was placed on the auricle and one on the ventricle in the following reproductions.

tered. I intend more explicitly to explain here the following details, which, in my opinion, are of interest for my subject.

1. As I indicated in my former investigations the a-v interval increases during the normal ventricle rhythm till the halving of the ventricle rhythm sets in. Afterwards the duration of the a-v interval decreases. The suspension curves of this figure show a much shorter a-v interval after the halving than before it. But the electrograms indicate these differences much sharper. The P-R interval increases still during the last four systoles. The first curve of the halved ventricle rhythm shows a much shorter P-R interval of the normal ventricle rhythm. The restoration of the ventricle muscle in the halved rhythm is even distinctly to be seen in these three first curves of the halved ventricle rhythm. The P-R interval of the second systole is shorter than that of the first, and that of the third still shorter than that of the second. We must attribute the shortening of the P-R interval after the halving to a shortening of the electric latent stage, as all sinus impulses reach the ventricle along the connecting systems and consequently the time of conducting along these has not in the least changed. It appears that this shortening still proceeds from the moment of the first ventricular systole of the halved rhythm.

2. The duration of the R-oscillation is, after the halving, shorter than before it; at the same time the height of the T-oscillation has increased. This duration of the R-oscillation is now, also, again shorter during the second systole than during the first, and at the third systole shorter than at the second. In concurrence with these facts the height of the T-oscillation increases from the first systole of the halved rhythm to the last one of the figure.

In the halved ventricle rhythm the conductivity through the ventricle is consequently better than in the normal twice-as-rapid rhythm of the ventricle. From the first systole of the halved ventricle rhythm, the conductivity still improves from systole to systole. The P-R interval and the duration of the R-oscillation consequently sustain alterations in exactly the same sense. We must attribute both these alterations to the changed metabolic condition of the ventricle muscle (katabolic index). This metabolic condition deteriorates in the normal ventricle rhythm. If now the rhythm of the ventricle suddenly halves the metabolic condition of the ventricle-muscles suddenly improves much, but also in the halved ventricle rhythm this improvement increases from systole to systole.

So this abrupt transition from the normal ventricular rhythm to the halved rhythm has originated in the following way. During the normal ventricular rhythm the duration of the residual refractory stage increases through accumulation from systole to systole. In the end the duration of the total refractory stage exceeds that of one sinus period. Then the next auricular systole is not followed by a ventricular systole and a prolonged ventricular pause arises. During this pause the duration of the residual refractory stage diminishes. This, of course, shortens the total refractory stage. But, at the same time, another influence is at work, which lengthens the duration of the total refractory stage. Its other component, viz., the periodic refractory stage increases in duration after the long ventricular pause. For after this pause the contractility of the ventricle augments. Now when, as in the present case, the increase of the duration of the periodic refractory stage exceeds the decrease of the duration of the residual refractory stage, the abrupt transition of the normal ventricular rhythm to the halved rhythm is brought about. This reproduction, which for the present moment will remain most likely exceptional among my material, afforded me an irrefutable confirmation of the theories I explained before. For the present I shall most likely be compelled in my further investigations to restrict myself to artificial transitions of poisoned frogs' hearts, and, when doing so, I shall, at the same time, register the action currents.

I am likewise in possession of beautiful examples of the slow transition to the halved rhythm of unpoisoned frogs' hearts. One example of these is reproduced in the figures 2, 3, 4 and 5.

The heart of a rana temporaria was suspended and soon showed group formation, because constantly one systole of the ventricle fell away. The groups grow gradually smaller, till groups of two and three systoles (figure 4) form the last transition to the halved ventricle rhythm (figure 5). We see during the groups the duration of the a-v interval increasing splendidly; again and again the ventricular systole sets in later in the auricular diastole, till one ventricular systole falls away. After this the interval is shortened again, to be protracted again in the same way during the following group. The ventricular systole of each first curve of the group commences in the figures 2, 3 and 4 close to the top of the auricular curve. The ventricular systole of each last curve begins at about the middle of the diastolic line of the auricular curves. This is the case with the large groups, but also with the little ones (bigeminus groups). Consequently in the beginning

more systoles of the ventricle are required than later to protract the a-v interval as much. The deterioration of the metabolic condition of the ventricle muscle is announced here by the formation of smaller groups. It is likewise clear that during the groups the metabolic condition of the ventricle muscle deteriorates, and improves again after

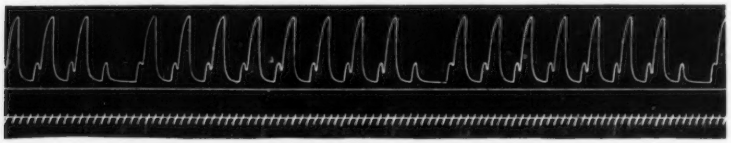

Fig. 2

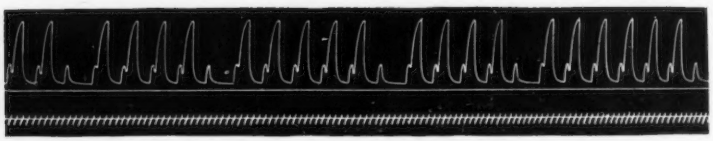

Fig. 3

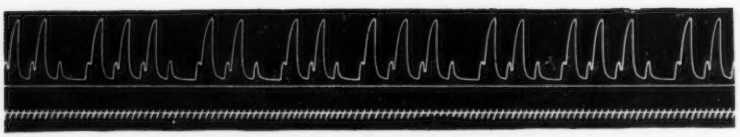

Fig. 4

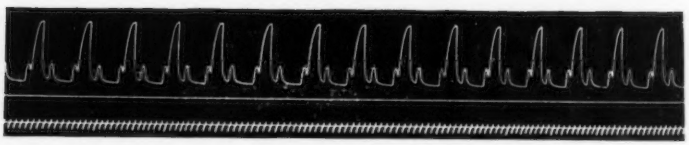

Fig. 5

a protracted pause. In my opinion we must here also attribute the protraction of the a-v interval again to a protraction of the latent stage of the ventricle muscle. It is the active contracting terminal organ, the ventricle muscle, the refractory stage of which increases during the groups and so does, at the same time, likewise the mechanical

latent stage. The increase of the refractory stage is here likewise caused by the increase of the duration of the residual refractory stage by accumulation. During the protracted pause after a group the decrease of the residual refractory stage surpasses the increase of the periodical refractory stage. In this way the constantly decreasing groups come into existence, which ends in the halved ventricle rhythm.

ARTIFICIAL CHANGES OF RHYTHM IN THE BLED FROG'S HEART

As has been shown in the preceding section, the halved ventricular rhythm may appear spontaneously in the bled frog's heart. This change may occur as soon as the duration of the total refractory stage of the ventricle exceeds the duration of one sinus period. Before the halved rhythm reveals itself spontaneously, we can halve the rhythm of the ventricle artificially, as appears from the following considerations. We call

$$\frac{\text{the duration of the total refractory stage}}{\text{the duration of a sinus period}}$$

the relative duration of the refractory stage.

When considering this fraction more carefully, we can say beforehand in what way the normal rhythm can be changed into a halved rhythm and the reverse, for if we take the relative duration of the refractory stage larger than one, the ventricle will pulsate in the halved rhythm. If, on the contrary, we take it smaller than one the ventricle will beat in the normal rhythm, in which every sinus impulse is followed by a systole of the ventricle. We can make the fraction greater than one by increasing the numerator or also by lessening the denominator. Now, in the case of a heart of which the total refractory stage is lengthened and which still beats in the normal rhythm, we can indeed prolong the total refractory stage so much as to make it outlast the sinus period. So we can make the fraction greater than one, as we have only to evoke an enlarged systole, whose refractory stage has been prolonged.

Now such an enlarged systole is the postcompensatory systole. When, therefore, we have lengthened the refractory stage of a ventricle (through poisoning or through bleeding) we evoke an extra-systole or extrapause of the ventricle. After the compensatory pause or extrapause the next ventricular systole is enlarged, while its refractory stage has been lengthened. Therefore, the subsequent sinus impulse

will be checked by this prolonged refractory stage; again, a prolonged pause ensues, and after this the next ventricular systole is again enlarged and has a prolonged refractory stage with all its consequences. Thus the ventricle is caught in the halved rhythm by the enlarged and broadened postcompensatory systole.[3] An increase of the duration of the refractory stage, i.e., an increase of the numerator of the above-mentioned fraction sufficed to bring about the ventricular halved rhythm. Another method producing the same result is heating the sinus venosus, which will increase the frequency of the sinus impulses and consequently decrease the duration of the sinus periods. The denominator of the fraction is diminished. When the ventricle pulsates in the halved rhythm, the relative duration of the refractory stage is greater than one. The fraction may then be made smaller by decreasing the numerator or by increasing the denominator.

The first may be effected by administering an extra stimulus to the ventricle during the diastole. Then an extra systole of the ventricle originates, which lasts much shorter than the ventricular systole from the ventricular halved rhythm. Therefore, the duration of its refractory stage is shortened and consequently the subsequent sinus impulse can elicit a ventricular systole. Owing to the short duration of the preceding ventricular pause, this systole will also be short, and accordingly will have only a short refractory stage. Therefore, here also the next sinus impulse is followed by a systole of the ventricle. Thus the ventricular halved rhythm is changed into the normal rhythm of double velocity. The extra stimulus during the halved rhythm may, however, be administered toward the end of the pause instead of during the diastole. Then the next sinus impulse reaches the ventricle during the diastole of the extrasystole and elicits a small ventricular systole. Whereas in the first case the normal ventricular rhythm was initiated by a small extrasystole, there now appears the normal rhythm under the influence of a sinus impulse, which reaches the ventricle in the diastole of an extrasystole and, therefore, yields a small systole. In both cases it was a *small ventricular systole with a short refractory stage,* that made the normal rhythm possible.

In the second place we can change the halved rhythm into the normal rhythm of twice its velocity by cooling the sinus venosus. Then the tempo of the sinus impulses is slackened by which the sinus periods are

[3] Not every postcompensatory systole is followed by a ventricular halved rhythm. This happens only when the refractory stage has been lengthened before by a disturbance of the metabolic equipoise.

lengthened. We will elucidate some of the above artificial changes of rhythm by some results obtained in experiments with the bled frog's heart.[4]

Let us first look at figure 5 of the previous publication.[5] The stimulating electrode is applied in the auriculo ventricular groove. At the downward deflection of the signal the ventricle receives a closing induction shock, which engenders an extra systole. At the end of the diastole of the postcompensatory systole, which has been enlarged, an opening induction shock is administered, which results in an extrapause of the ventricle.[6]

After the extrapause the first ventricular systole has increased still more in magnitude and in breadth, so that now the next sinus impulse rebounds on the refractory stage. The subsequent prolonged ventricular pause again causes an enlarged ventricular systole with a prolonged refractory stage.

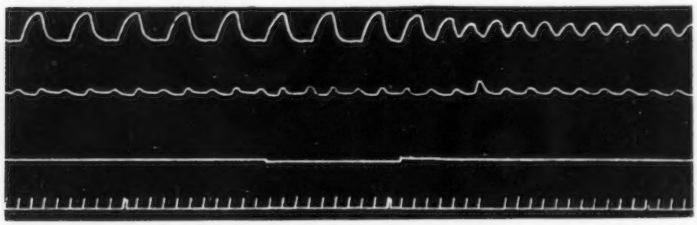

Fig. 6

Again the next sinus impulse does not result in a ventricular systole. Thus the ventricle, pulsating in the halved rhythm is, so to speak, caught in its own rhythm through the prolonged refractory stage. We can change this halved rhythm again into the normal rhythm of twice the rapidity, by eliciting a small ventricular systole. This happens in figure 6.[7] At the downward deflection of the signal an auricular extrasystole was evoked, after which the excitation reached the ventricle during the refractory stage. Consequently the rhythm of the ven-

[4] In all the figures of this publication the upper row represents the suspension curves of the ventricle, the lower row the suspension curves of the auricles.

[5] S. de Boer: On the artificial extra-pause of the ventricle of the frog's heart. This Journal.

[6] For the causes of this extra-pause I refer to the previous publication.

[7] Between figure 5 of the previous publication and figure 6 of this paper two ventricular systoles have not been reproduced.

tricle did not change here. However, at the upward deflection of the signal the stimulus was repeated toward the end of the pause. Now the auricles are refractory, but the ventricle responds to the stimulus with an extrasystole. After this the periodic sinus impulse reaches the ventricle at the end of the diastole so that a decreased systole of the ventricle ensues. This is accompanied by a short refractory stage so that also the subsequent sinus impulse again results in a ventricular systole. In this way every sinus impulse may be followed by a ventricular systole.

Figure 7 shows the suspension curves of a frog's heart, ten minutes after bleeding. The stimulating electrode is at the auricles. At the first downward deflection of the signal the auricles receive a closing shock which results in an extrasystole of the auricles followed by a compensatory pause.

It is evident that the ventricular rhythm is influenced only in this way that the next systole of the ventricle appears somewhat earlier.

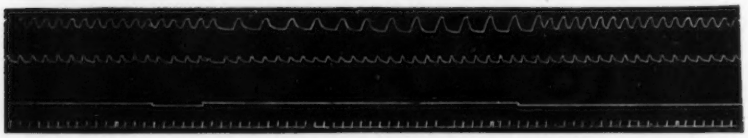

Fig. 7

When, however, at the upward deflection of the signal the auricles receive the opening induction shock at an earlier moment of the auricular period, the result is quite different. After the thus excited extrasystole of the auricles, coinciding with the commencement of the ventricular systole, the excitation reaches the ventricle still in the latter's refractory stage.

After the compensatory pause of the auricles the next auricular systole is followed again by a ventricular systole. Thus arises an extrapause of the ventricle followed by an enlarged and broadened systole. Of this the refractory stage is prolonged, so that the next auricular systole cannot be followed by a ventricular systole. Again a prolonged ventricular pause arises, which is again followed by an enlarged systole of the ventricle. Thus the ventricle is caught in the halved rhythm by only one stimulus administered to the auricles. At the second downward deflection of the signal the auricles receive a closing shock toward the close of the pause, which evokes an extrasystole of these chambers. After this the next ventricular systole commences earlier.

The then following sinus impulse reaches the ventricle toward the close of the diastole and may,therefore, be followed by a small ventricular systole. This small ventricular systole now yields a short refractory stage. Therefore, the next auricular systole can be followed again by a ventricular systole, which, on account of the short duration of the preceding pause, is again small and short. For this reason the next auricular systole can again be followed by a ventricular systole. Thus by a single induction shock the halved rhythm of the ventricle is changed into the normal rhythm of twice the rapidity. Figures 6 and 7 show us that we are able to change the halved rhythm into the normal one by means of a single induction shock. Now the question arises why the ventricle does not take up the normal rhythm spontaneously. From the fact that the halved rhythm can be changed into the normal it indeed appears that the metabolic condition of the ventricular muscle enables the ventricle to beat with a double frequency. Still the ventricle persists in its halved rhythm unless we administer a stimulus at the right moment. The cause must be looked for in the magnitude and the long duration of the ventricular systoles of the halved rhythm. Every second sinus impulse rebounds on this prolonged refractory stage; the ventricle is caught in the halved rhythm and can escape from it only when, through an extra stimulus a small ventricular systole is evoked directly or indirectly.

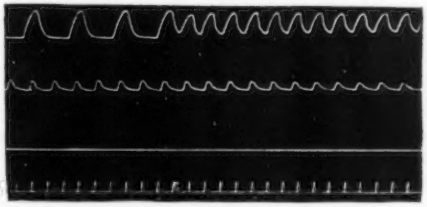

Fig. 8

When, however, the ventricle has been pulsating for some time in the halved rhythm, the ventricle gradually discards the residual refractory stage under the influence of the many prolonged ventricular pauses so that the total refractory stage is shortened after all. In this way the normal ventricular rhythm may yet return spontaneously. This is illustrated in figure 8.

The curves of this figure originate from the same frog's heart which produced the curves of figure 7.

When looking again at the ventricle curves of figure 5 of the previous publication and of figures 6, 7 and 8 of the present one we can state what follows:

As soon as the normal ventricular rhythm is changed into the halved rhythm the magnitude and the duration of the ventricular systole in-

creases. This increment then proceeds from systole to systole, so that the tenth systole of the halved rhythm is much greater than the fifth, which again in its turn is greater than the first. This increment of the magnitude of the ventricular systole is brought about by an increase of the maximum diastole and, at the same time, by an increase of the maximum systole. It will be seen, then, that the ventricular muscle recovers during the halved rhythm and that this recovery proceeds under the influence of an increase of long ventricular pauses. The reverse will be observed after the change of the halved rhythm into the normal. The ventricle is then in a good condition owing to the preceding halved rhythm. Directly after the change into the normal rhythm, the magnitude of the ventricular systoles has decreased. But under the influence of the frequent recurrence of short ventricular pauses the magnitude of the ventricular systoles lessens more and more. This lessening regards the maximum diastole as well as the maximum systole. An intermediate form between the normal ventricular rhythm and the halved rhythm is the ventricle alternation.

We can change the normal ventricular rhythm into the alternation and this again into the halved rhythm, as illustrated in the following figures, derived from the same frog's heart. The curves of figure 9 were taken five minutes after the bleeding. The ventricle was then pulsating in the normal rhythm; at the first deflection of the signal the auricles received an induction shock resulting in an extrasystole of these chambers which was followed by a small systole of the ventricle. At the second deflection of the signal again an auricular extrasystole was evoked in the beginning of the postcompensatory systole. Thereafter the excitation reaches the ventricle during the refractory stage so that an extrapause of the ventricle ensues. Then the first ventricular systole is very much enlarged. This enlarged ventricular systole introduces an alternation of the ventricle. (Similarly in our previous experiments the halved rhythm was brought about by an enlarged systole.) After some time this alternation changes spontaneously into the normal ventricular rhythm with systoles of the same magnitude. At the third deflection of the signal again an extrasystole of the auricles is evoked, followed by a small ventricular systole. After the enlarged postcompensatory systole again the ventricle alternation arises.

The curves of figure 10 were taken about one minute after those of figure 9, a short time before the alternation had been elicited experimentally. It still exists at the commencement of the figure. At the first deflection of the signal the auricles are excited to an extrasystole

by an induction shock in the beginning of a large ventricular systole. After this extrasystole the excitation reaches the ventricle during the refractory stage so that no ventricular systole follows; an extra pause of the ventricle does follow. After this extrapause the first ventricular systole is enlarged again so that the next sinus impulse reaches the ventricle during the refractory stage. Owing to this the next pause of the ventricle is again prolonged with the ordinary consequences. In this way the ventricular halved rhythm is brought about artificially.

At the second deflection of the signal again an extrasystole of the auricles is evoked in the beginning of a ventricular systole. Because

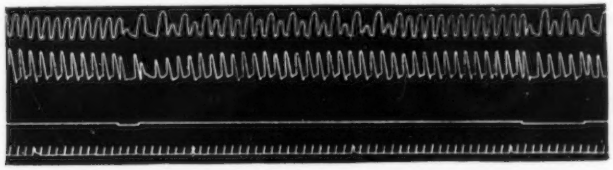

Fig. 9

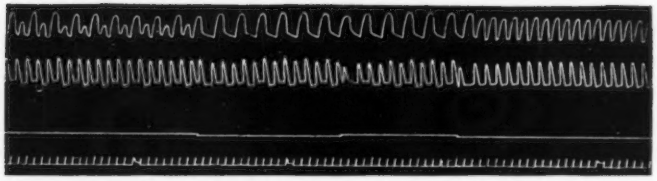

Fig. 10

hereafter the excitation reaches the ventricle during the refractory stage, the halved rhythm of course continues.

At the third deflection of the signal, however, an extrasystole of the auricles is evoked after the close of a ventricular systole. After this the excitation reaches the ventricle toward the end of the pause so that a premature ventricular systole follows. Now because this ventricular systole is premature the next sinus impulse reaches the ventricle after the close of the refractory stage, so that a small systole of the ventricle can follow. This systole is small on account of the short duration of the preceding pause and, therefore, causes a short refractory stage. For this reason also the following sinus impulse is

again followed by a ventricular systole, which also is small. In this way the normal rhythm of the ventricle is restored.

In the above we have given some instances of changes of rhythm in the bled frog's heart. We could enforce at will any given rhythm upon the ventricle by evoking *one* ventricular systole of a certain magnitude and duration.

ARTIFICIAL AND SPONTANEOUS CHANGES OF RHYTHM WITH SIMULTANEOUS REGISTRATION OF THE ELECTROGRAMS

It is exceedingly interesting to register cardiac electrograms during the artificial and spontaneous changes of rhythm. It affords us an opportunity of studying in the ventricular electrograms of one and the same frog's heart, the ventricular beats which occur after long and after short ventricular pauses. The influence of the duration of the pause immediately preceding on the form of the ventricular electrogram will appear from what follows:

A. Influence of the velocity of the conduction of excitation wave on the form of the ventricular electrogram. In a previous publication (2) I have set forth what was the influence of the velocity of the conduction of the excitation wave through the ventricle on the form of the ventricular electrogram. I could do this by slowing the conduction of the excitation wave in the same frog's heart. I thus obtained of one and the same frog's heart ventricular electrograms with rapid and with slow conduction of the excitation wave through the ventricle. Our procedure was threefold:

1. In the first series of experiments the electrograms of each frog's heart were registered while the circulation of the blood was left unimpaired. Subsequently a toxic dose of digitalis or of antiarin was given subcutaneously. With intervals of some minutes the electrograms were taken, until the ventricular rhythm began to be halved. Under the influence of the drug the velocity of the conduction of the excitation wave through the ventricle lessened. This led to the following alteration of the electrogram: α The R-deflection got broader; β the T-deflection was altered in a negative sense (a positive T-deflection became smaller, or changed into a negative one, a negative T-deflection became larger); γ the connecting line between the R-, and the T-deflection was lowered. These three changes made themselves more evident as the poisoning proceeded, i. e., as the velocity of the conduction of the excitation wave through the ventricle decreased more and

more. Then the rhythm of the ventricle was halved. During the halved ventricular rhythm the ventricular pauses have been enlarged. For this reason the excitation wave is sent through the ventricle at a quicker rate. This acceleration manifests itself directly in the form of the ventricular electrogram. The R-deflections narrow, the T-deflections are modified in a positive sense (the negative T-deflections become smaller or turn into positive T-deflections), the connecting lines between the R- and the T-deflections rise.

2. In the second series of experiments the normal ventricular rhythm was, after poisoning with digitalis or antiarin, transposed through one induction shock to the halved rhythm and this again to the normal one. In this way I procured records of the halved and the normal ventricular rhythm of the same heart. At the same time the electrograms were registered to the following effect:

When the normal ventricular rhythm was transposed to the halved rhythm, the R-deflections were narrowed, the T-deflections were modified in a positive sense and the connecting lines between the R- and the T-deflections rose. If, on the contrary, the halved rhythm was changed into the normal one the R-deflections broadened again, the T-deflections changed in a negative sense and the connecting lines between the R- and the T-deflections were lowered. These two series of experiments go to show distinctly that a slowing of conduction of the excitation wave through the ventricle causes the following regular and typical changes in the ventricular electrogram; the R-deflection broadens; the T-deflection changes in a negative sense; the connecting line between the R- and the T-deflections falls. A clearer view still was given of these typical changes in the third series of experiments.

3. In this series we experimented on non-poisoned frog's hearts. Ventricular extrasystoles were excited in a more or less premature stage of the ventricular period. The earlier a ventricular extrasystole was incited in the ventricular period, the slower was the conduction of excitation through the ventricle during the extrasystole. The electrograms of the frog's hearts were registered simultaneously. Now, when the extra shock was administered to the basis of the ventricle an electrogram resulted, of which the R-deflection was broadened, the connecting line between the R- and the T-deflections was lowered and the T-deflection had been changed in a negative sense. These modifications manifested themselves all the more distinctly according as the extra shock had been administered at an earlier stage of the ventricular period. It goes without saying that here we can only compare the

electrograms of the more or less premature extrasystoles among themselves, and not with those of the periodic ventricular systoles, in order to realize the influence of the velocity of the conduction of the excitation wave on the form of the ventricular electrogram. For with the former the induction shock affects the ventricle at a special point of the exterior muscular layer and with the latter the excitation wave proceeds along the atrio-ventricular connecting system. If, therefore, we wish to know the influence exercised by the velocity of the conduction on the form of the ventricular electrogram we can compare the electrograms of ventricular extrasystoles, which have been incited at an early stage of the ventricular period with those of the ventricular extrasystoles that have been incited later. The result was that with the former the R-deflection had been broadened more, the connecting line between the R- and the T-deflections had fallen lower, the T-deflection had undergone a greater change in a negative sense, than with the latter. These changes, therefore, will make themselves more evident according as the extrasystole was incited at an earlier moment of the ventricular period.

Now when we administer an induction shock at the apex, the excitation wave traverses the ventricle during the subsequent extrasystole from the apex to the base. This circumstance causes the R-deflection to revert, as already established by Samojloff (3). The R-deflection has also broadened and the more so according as the induction shock occurred earlier in the ventricular period, i.e., according as the excitation wave proceeded through the ventricle at a slower rate. The T-deflection has changed in a positive sense (a negative T decreased or turned into a positive one, a positive T increased) and that the more intensely, according as the induction shock was given at an earlier moment of the ventricular period. The connecting line between the R and the T has risen, and that all the more according as the induction shock had been administered at an earlier moment of the ventricular period.

In the foregoing we have compared the electrograms of the extrasystoles after base and apex-stimulation with the electrograms of the periodic ventricular systoles. However, in order to observe the influence of the velocity of conduction of the excitation wave on the form of the ventricular electrogram we compared, after base or apex-stimulation, the electrograms of the extrasystoles among themselves, which had been incited at an earlier or later moment of the ventricular period. In the experiments I undertook by means of stimulation of the auricles,

I also could compare the electrograms of the subsequent premature ventricular systoles with each other and also with those of the periodic ventricular systoles. For when an auricular extrasystole has been incited, the excitation wave will proceed along the atrio-ventricular connecting systems to the ventricle and will enter the ventricle at the same points as with the periodic ventricular systoles.

From these experiments it appeared that after extra-stimulation of the auricles the electrograms of the subsequent ventricular systoles exhibited the following alterations:

1. The R-deflections had been broadened.
2. The T-deflections were changed in a negative sense.
3. The connecting lines between the R- and the T- had been lowered.

These alterations became more distinct according as the ventricular systole appeared at an earlier moment of the ventricular period, i.e., according as the excitation wave was conducted through the ventricle at a slower rate.

The electrograms of the postcompensatory systoles appearing after base, apex or auricle stimulation, are also essential to our subject. After the lengthened compensatory pause the excitation is accelerated during the postcompensatory systole on traversing the ventricle. Owing to this the electrograms of the postcompensatory systoles undergo the following alterations:

1. The R-deflections have narrowed.
2. The T-deflections have changed in a positive sense.
3. The connecting lines between the R- and the T-deflections have risen.

These changes are revealed more distinctly according as the compensatory pause lasts longer, i.e., according as the preceding extrasystole of the ventricle is incited at an earlier moment of the ventricular period. The rate of conduction of excitation during the postcompensatory pause is the greater the sooner the preceding extrasystole of the ventricle has been incited in the ventricular period. It will be understood that the first two series of experiments (intoxication with antiarin or digitalis) were performed on frogs' hearts that were still perfused with blood. Their results are therefore not quite reliable, since the varying infusion of blood may affect the form of the ventricular electrogram.

In the third series of experiments we used bled frogs' hearts as well as those in which the blood-circulation was left unimpaired. The changes described above occurred in either case. This result also

lent support to the experiments of the first two series. The joint results of the three series of experiments may, therefore, be summarized as follows:

Slowing of the conduction of the excitation wave through the ventricle causes:

1. The R-deflection to be broadened.
2. The T-deflection to be changed in a negative sense.
3. The connecting line between R and T to be lowered. These changes will be greater according as the excitation wave is conducted through the ventricle at a slower rate. Acceleration of the conduction of the excitation wave through the ventricle causes.

1. The R-deflection to be narrowed.
2. The T-deflections to be changed in a positive sense.
3. The connecting line between R and T to rise. These changes will be greater according as the excitation wave is conducted through the ventricle at a quicker rate.

Why these changes influence the form of the ventricular electrogram when the conduction of the excitation wave is slowed or accelerated, we can elucidate best by some diagrams.

Figure 11 illustrates a ventricular electrogram with a positive T-deflection. This electrogram is the product of interference of the basal negativity *a*, *b*, *c*, with the apical *e*, *f*, *g*. The apical negativity commences at *e*, by which the initial basal deflection is compensated. Thus arises the R-deflection. After this the two negativities counterbalance each other, when finally the positive T-deflection appears because the basal negativity outlasts the apical.

Now when the velocity of the conduction of the excitation wave through the ventricle diminishes, the apical negativity commences later after the commencement of the basal. The point *e* gets farther removed from *a*. The entire apical negativity is thrust over and even past the basal, so that at the end the apical negativity is still persisting, when the basal negativity has already terminated. The T-deflection is consequently negative[8] (see fig. 12). Before the slowing (see fig. 11) point *n* of the basal negativity interfered with point *n'* of the apical. These two points being equidistant from the position of rest, their resultant coincides with the position of rest. But after the slowing *n* interferes with *m'* which is farther from the position of rest, so that the resultant is seen to lie below the position of rest. After the slowing

[8] It is obvious that the positive T. is diminished when the slowing of the conduction of excitation wave is less pronounced.

this holds good for all points of the basal negativity curves, which then interfere with points of the apical negativity curve, which are all farther away from the position of rest. In consequence of this the connecting line between R and T is lowered (fig. 12). When, on the contrary, the velocity of the conduction of the excitation wave increases, *e* gets nearer to *a*, and the curve of the apical negativity shifts in the opposite direction, so that after the conclusion of the apical negativity the basal negativity continues. This causes an enlargement of the positive T-deflection. After this acceleration every point of the basal negativity curve interferes with a point of the apical negativity curve, which lies nearer to the position of rest, so that the connecting line between R and T rises. (See fig. 13.)

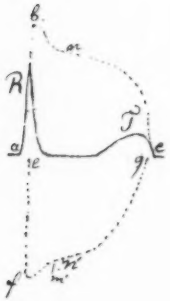

Fig. 11

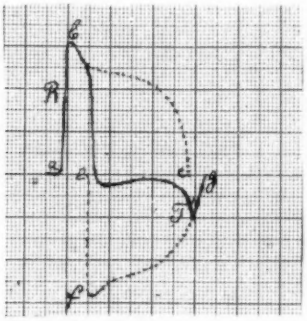

Fig. 12

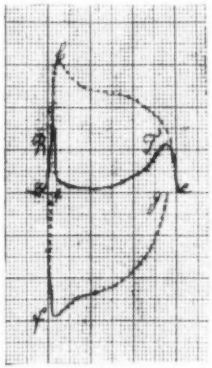

Fig. 13

After this investigation, which was in part carried out with poisoned frogs' hearts with unimpaired circulation some interest hinged about the study of the form of the electrogram of bled frogs' hearts, exhibiting analogous rhythmic disturbances. We will illustrate this by the following example:

Figure 14 shows the suspension curves (double suspension) and the electrograms of a bled frog's heart. The upper curves are derived from the suspended ventricle; then follow the auricle curves and under these the electrograms (derivation auricle-ventricular apex, tension of the string is such that 1 mv. yields a deflection of $1\frac{1}{2}$ mm.). In the upper illustration every third auricular systole is not followed by a systole of the ventricle, so that groups of two ventricular systoles are

engendered (at the end a group of three ventricular systoles can be seen). The first ventricular systole of every group appears after a long pause, the second after a short one, from which we see that during the first ventricular systole of every group the excitation wave traverses the ventricle at a quicker rate than during the second. This is brought out distinctly in the ventricular electrograms of the groups. The R-deflection of every second electrogram is much broader than that of every first, whereas the R-deflection of every second electrogram rises very slowly, that of every first electrogram rises abruptly.

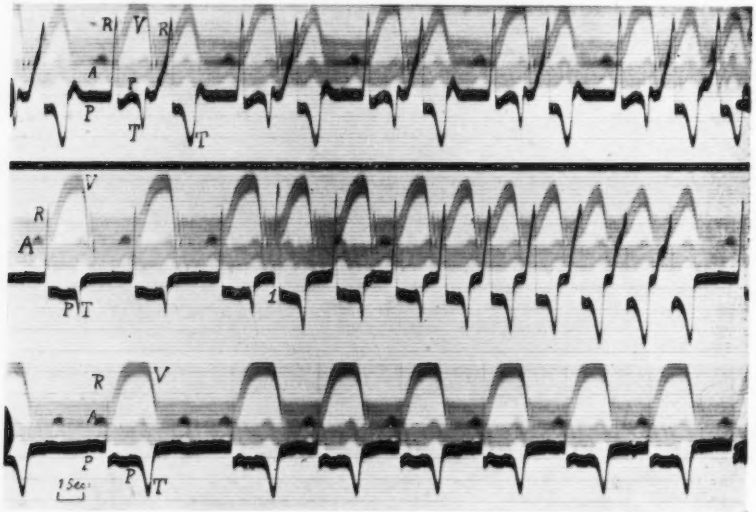

Fig. 14

The negative T-deflections of every second electrogram are much larger than of every first, and the connecting line between R and T is lower for every second electrogram than with every first.[9] Also the

[9] After every second ventricular electrogram occurs an approximately triangular upward deflection. Most likely this is the electric equivalent of an extremely small abortive third ventricular contraction. This view is favored by the following data: 1, these deflections commence after the conclusion of every third auricular systole; 2, now the deflections are more extensive, now again somewhat smaller, since now a larger, now again a smaller portion of the ventricular muscle is contracted; 3, in the trigeminus group at the end of the row the third R-deflection begins with such a triangular curve. The little positive peaks at the end of every first electrogram are of the same magnitude and occur no doubt with every first ventricular electrogram.

curves of the middle illustration are remarkable. Initially the ventricle pulsated in the halved rhythm so that with every two auricular systoles there is one ventricular systole. At *1* an induction shock was administered to the ventricle which generated an extrasystole, which is succeeded by a second. Now because the last mentioned extrasystole commences at an earlier moment of the ventricular period (directly after the P-deflection) it is only of short duration. The refractory stage of the extrasystole is also of shorter duration than that of any preceding ventricular systole of the halved rhythm. This is why the next auricular systole can be followed again by a systole of the ventricle, which in consequence of the short duration of the preceding ventricular pause is very brief again and is accordingly accompanied by a brief refractory stage. Therefore, also, now the next auricular systole can again be followed by a systole of the ventricle. Thus the halved rhythm is transposed by *one* induction shock to the normal rhythm of double velocity. This normal rhythm maintains itself during eight systoles and then again changes spontaneously into the halved rhythm of the ventricle. This transition takes place because during the normal ventricular rhythm the ventricle cannot restore itself sufficiently in the brief ventricular pauses. So a residual refractory stage remains after every ventricular systole, so that after eight ventricular systoles this residual refractory stage has been prolonged to such an extent that the duration of the total refractory stage exceeds the duration of a sinus period. In that case the next auricular systole is not followed by a systole of the ventricle, and a lengthened ventricular pause is brought about. After this pause the first ventricular systole is enlarged, it lasts longer and has a long refractory stage. The subsequent auricular systole can, therefore, not be succeeded by a systole of the ventricle and again a prolonged ventricular pause arises with all its consequences. Thus the halved rhythm reveals itself again spontaneously.[10]

A comparison of the ventricular electrograms of the two ventricular rhythms is now of interest for our subject. It is obvious that during every ventricular systole of the halved rhythm the excitation wave traverses the ventricle at a quick rate under the influence of the long ventricular pauses. During the normal ventricular rhythm the ventricular pauses are of short duration, so that the excitation wave traverses the ventricle slowly. The ventricular electrograms of the halved rhythm display narrow R-deflections, the T-deflections

[10] In the figure we see only the R-deflection of the first systole of the halved rhythm.

are negative, the connecting lines between the R and T are lying below the position of rest. The ventricular electrogram of the first systole of the normal rhythm displays a broadened R-deflection, the negative T-deflection is enlarged and the connecting line between the R- and the T- has been slightly lowered. After this the R-deflection broadens from systole to systole; the longer the normal rhythm of the ventricle maintains itself the slower is the rise of the R-deflections. In addition to this the negative T-deflections are getting larger and larger; the connecting lines are lowered more and more every time. So during the normal ventricular rhythm we see that the velocity of the conduction of the excitation wave decreases from the first to the eighth systole. The larger the number of preceding brief ventricular pauses, the slower is the rate at which the excitation wave traverses the ventricle.

During the halved ventricular rhythm the apical negativity surpassed already the basal negativity at the end of the electrogram. Therefore, already here, the T-deflection was negative. But if the velocity of the conduction of the excitation wave through the ventricle diminishes increasingly, the apical negativity continues longer and longer after the conclusion of the basal negativity. Thereby the ventricular electrogram is broadened more and more, and an increasingly greater part of the apical negativity still persists after the conclusion of the basal. This induces a longer duration of the ventricular electrograms. The last ventricular electrogram of the normal ventricular rhythm lasts much longer than the first. Since the frequency of the sinus impulses remains the same, the electric ventricular pause decreases progressively. It can be seen, therefore, that during the normal ventricular rhythm, the time during which the string remains in the position of rest between two electrograms is becoming shorter every time. Together with the progressive decrease of the velocity of the conduction of the excitation wave in the normal ventricular rhythm, this figure also shows a progressive decrease of the contractility of the ventricular muscle. Both the progressive decrease of the contractility and the progressive decrease of the rate of the conduction of the excitation wave are a direct consequence of the progressive deterioration of the metabolic condition of the ventricle. The greater the number of preceding brief ventricular pauses, the worse the metabolic condition becomes. We have also observed heretofore that after the transition from the normal ventricular rhythm to the halved rhythm, the contractility of the ventricle in this halved rhythm increases from systole

to systole. Therefore several long ventricular pauses are wanted to make the ventricle resume its contractility entirely. This also seems to be the case with regard to the velocity of the conduction of excitation, for if we consider the R-deflection of the first ventricular systole of the returned halved rhythm more narrowly, it is evident that this deflection has indeed become much narrower than that of the preceding normal ventricular rhythm. The immediate recovery of the rate of the conduction of the excitation wave through the ventricle after one long ventricular pause is, therefore, not questionable. Besides also the R-deflection rises quickly. Now in comparing the breadth of this R-deflection with that of the R-deflections of the halved ventricular rhythm with which this registration began, it appears that this R-deflection is no doubt about twice or three times as broad. It is evident, therefore, that after this single prolonged ventricular pause the velocity of the conduction of the excitation wave has by no means regained its optimum. It should seem, therefore, that for this as for the contractility several prolonged ventricular pauses are required. That after one prolonged ventricular pause the conduction of the excitation through the ventricle cannot recover its optimum, the upper illustration tends to show. After a prolonged ventricular pause the R-deflection of the first systole of every group has, indeed, become narrower, but is of about the same breadth as the last R-deflection of the second illustration. Here we fail to see after one prolonged pause a recovery of conductivity up to its optimum. From these two illustrations it is evident that in consequence of a slowing of the conduction of excitation through the ventricle:

1. The R-deflection broadens.
2. The T-deflection is modified in a negative sense.
3. The connecting line between the R and the T is lowered. These modifications will become more pronounced, according as the velocity of the conduction of excitation through the ventricle diminishes.

Hofmann (4) and Mines (5) believe that the duration of the ventricular electrograms affords an index of the contractility. The latter increases with a longer duration of the electrogam. Now, when measuring the curves of figure 14 we arrive at another conclusion. In the sets of two curves of the upper row the contraction of every first ventricular curve is the greatest, but the duration of the electrogram of every second curve far exceeds that of every first. A similar result is seen when measuring the second row of curves. In them the contractility decreases considerably during the normal ventricular

rhythm but the duration of the ventricular electrograms increases. Nay, the duration of the last ventricular electrogram even exceeds that of the ventricular electrograms of the halved ventricular rhythm with which this row commences. It follows that we cannot take the duration of the ventricular rhythms as an index of the contractility. The duration of the ventricular electrograms of every second curve of the groups of the upper row has increased so much because the velocity of the conduction of excitation through the ventricle has decreased considerably. Consequently the apical component of the ventricular electrogram overlaps the basal so that the electrogram is broadened. The same holds for the ventricular electrograms of the normal rhythm of the second row of curves. The longer the normal ventricular rhythm maintains itself the more the velocity of the conduction of excitation through the ventricle will decrease, and the more the ventricular electrograms will broaden. The tempo of the auricular beats being constant, we can read the increase of the duration of the ventricular electrograms in the normal ventricular rhythm directly from the decrease of the duration of the electric pauses.

B. The optimum of velocity of the conduction of the excitation wave through the ventricle. The lowermost illustration of figure 14 begins with the 3–1 rhythm: with every three auricular systoles there is one systole of the ventricle. After two ventricular systoles this 3–1 rhythm changes spontaneously into the halved rhythm of the ventricle. Now, when comparing the suspension curves of the ventricle in these two rhythms with each other we see that the contractility in these rhythms does differ. It is well known already that with a certain frequency, after artificial stimulation of heart-preparations that have been standing for some time, the cardiac muscle attains the optimum of contractility. (Hofmann (4) Mines (5).) A decreased and an increased frequency produce a decrease of contractility. From the lowermost row of curves it appears that, for this heart, the optimum of frequency of the ventricle is not yet reached with the halved and probably with the 3–1 rhythm. Acceleration of the pulsations decreases the contractility as will be seen from the middle illustration.

In this figure no slowing of the ventricular pulsations occurs that exceeds the 3–1 rhythm. So nothing can be said about it regarding this heart. True, the optimum of frequency has been reached probably during the 3–1 rhythm.[11]

[11] The height of contraction in either (2–1 and 3–1) rhythm is the same, but the duration of a ventricular systole of the 3–1 rhythm is longer than that of the halved rhythm.

Now the ventricular electrograms show that during the halved rhythm the velocity of the conduction of excitation through the ventricle has already reached its optimum. The longer ventricular pauses during the 3–1 rhythm do not increase this velocity any more, since the R-deflections in either rhythm are of the same breadth, the T-deflections of the same magnitude and the connecting lines between R and T are in both rhythms on the same level. We see then that the optimum of the contractility does not coincide wholly with the optimum of the velocity of the conduction of excitation wave through the ventricle[12]

C. On the relationship between the mechanic latent stage of the ventricle and the velocity of the conduction of the excitation wave through the ventricle. Gad's researches (6) have established that the mechanic latent stage of skeletal muscle is principally due to the mechanic relations of the registration. This has been demonstrated by Gad in the following way: He tied together two frog's muscles, the one above the other, and attached the terminal tendon of each muscle to a lever. Now the nether muscle was stimulated by an induction shock, so that it began to contract. The bottom lever now registered a shortening. The top lever however registered synchronously a lengthening. This was because the top muscle was extended through the contraction of the nether muscle. This finding Gad applied to one muscle, for an interpretation of the mechanic latent stage. For, when a muscle is affected by an induction shock at a definite point, the wave of contraction begins at that point. A short time after, a definite part of the muscle is contracted. This contracting portion now extends the flaccid portion of the muscle. As soon as the contraction-wave has proceeded so far that the contracting part exceeds the relaxing portion, the lever rises and the mechanical curve comes forward. It is evident, therefore, that the mechanic latent stage results from the mechanic relations of the registration. We may conclude from this also that the mechanic latent stage will last the longer, according as the excitation proceeds through the muscle at a slower rate. Now I found the same relations as those for skeletal muscle to exist also for the heart. Let us consider the two upper rows of curves of figure 14.

For index of the mechanic latent stage of the ventricular muscle we take the R-V-interval, the time elapsing from the commencement of

[12] In figure 14 the lower most row of curves was registered first, namely, some minutes after the bleeding. Then the middle one and finally the upper row, every time with an interval of some minutes. During the lowermost registration the string was slightly restless owing to a little disturbance in the compensation current.

the R-deflection to the commencement of the suspension curve, for when the R-deflection begins the excitation wave has no doubt already reached the ventricle. For index of the velocity of the excitation-wave through the ventricle we take the breadth of the R-deflection. We shall, therefore, compare the breadth of the R-deflections and the duration of the R-V-intervals during the sets of two systoles of the upper row and at the same time compare those of the halved rhythm and the normal rhythm of the second row.

Upper row of curves

Duration of the R-deflections of every first ventr. syst. $= \frac{1}{3}$ sec.
Duration of the R-deflections of every second ventr. syst. $= \frac{2}{3}$ sec.
Duration of the R-V-interval of every first ventr. syst. $= \frac{1}{4}$ sec.
Duration of the R-V-interval of every second ventr. syst. $= \frac{1}{2}$ sec.

From which we see that during every first systole succeeding a lengthened ventricular pause, the excitation wave is conducted through the ventricle twice as rapidly as during every second systole.

In accordance with this also the latent stage in every first ventricular systole has twice the length of that in every second ventricular systole.

Second row of curves

Halved ventricular rhythm.
Duration of the R-deflections $= \frac{1}{6}$ sec.
Duration of the R-V-interval $= \frac{1}{6}$ sec.

Normal ventricular rhythm

Duration of the R-deflections from the first to the last systole $\frac{6}{24}$ sec., $\frac{7}{24}$ sec., $\frac{8}{24}$ sec., $\frac{10}{24}$ sec., $\frac{12}{24}$ sec., $\frac{15}{24}$ sec., $\frac{16}{24}$ sec.

Duration of the R-V-intervals: $\frac{6}{24}$ sec., $\frac{8}{24}$ sec., $\frac{9}{24}$ sec., $\frac{10}{24}$ sec., $\frac{11}{24}$ sec., $\frac{13}{24}$ sec., $\frac{19}{24}$ sec. We see then that during the normal ventricular rhythm the excitation-wave proceeds through the ventricle at a slower rate than during the halved rhythm of the ventricle. The duration of the R-deflections increases from the first to the last systole of the normal ventricular rhythm, consequently the velocity with which the excitation wave traverses the ventricle diminishes. Accordingly the mechanic latent stage is of longer duration during the normal ventricular rhythm than during the halved ventricular rhythm. From the first ventricular systole of the normal ventricular rhythm down to the last there is an increase of the duration of the mechanic latent stage.

It follows, then, that *the duration of the mechanic latent stage of the ventricle increases with a decrease of the velocity of the conduction of excitation through the ventricle.*

I have expounded the relation between the velocity of the conduction of the excitation wave through the ventricle and the duration of the mechanic latent stage of the ventricle on the basis of the example illustrated in figure 14. I always found this relation in my previous researches, when ceteris paricus the metabolic condition of the ventricular muscle altered. For this I refer the reader to figures 4 to 18 (inclusive) of my communication in Pflüger's Archiv der Physiologie Bd. 173, Seite 78. "Ueber den Einflusz der Geschwindigkeit der Reizleitung auf die Form der Kammerelectrogramme."

D. On the electric latent stage of the ventricle. As observed before (page 190), from the fact of the transposition by means of one induction-shock from the halved rhythm to the normal we conclude that during the halved ventricular rhythm, also every sinus-impulse, not responded to by the ventricle, reaches the ventricle along the atrio-ventricular connecting systems. Because all sinus impulses reach the ventricle, a lengthening of the P-R interval during the normal ventricular rhythm can not be ascribed to a lengthening of the time of conduction through the auricles and the atrio-ventricular connecting systems, but to a prolongation of the electric, latent stage of the ventricle.

However, during the normal ventricular rhythm the P-deflections coincide with the ventricular electrograms and have become invisible. We, therefore, determine the A-R intervals (the time elapsing between the commencement of the auricular systoles and the beginning of the R-deflections). Duration of the A-R interval during the halved ventricular rhythm = $\frac{1}{2}$ second. Duration of the A-R intervals during the normal ventricular rhythm: of the first ventricular systole = $\frac{1}{2}$ second, of the last ventricular systole = $\frac{9}{12}$ second. It is evident, therefore, that the electric latent stage of the ventricle during the halved ventricular rhythm is as great as during the first systole of the normal ventricular rhythm. From the first systole of the normal ventricular rhythm the electric latent stage of the ventricle increases down to the last systole. We see, however, that this increase ($\frac{1}{4}$ second) is much smaller than the increase of the mechanic latent stage ($\frac{1}{2}\frac{3}{4}$ second).

SUMMARY

The results of our investigation are as follows:

1. After bleeding the frog's heart the halved ventricular rhythm may come forth spontaneously. This transition may occur abruptly or gradually through group formation. The causes of sudden and slow transition were ascertained.

2. A short time before the ventricular rhythm is halved, the metabolic condition of the ventricular muscle is deteriorated. This deterioration reveals itself *a*, in a lengthening of the refractory stage; *b*, in a slowing of the conduction of the excitation wave through the ventricle; *c*, in a lengthening of the a-v interval; *d*, in a decrease of the contractility.

3. After the ventricular rhythm has changed into the halved rhythm the metabolic condition of the ventricular muscle improves under the influence of the lengthened ventricular pauses. However, owing to the increase of the contractility and the longer duration of the ventricular systoles, the duration of the refractory stage does not decrease. The excitation is led through the ventricle at a quicker rate, the a-v interval is shortened.

4. The halved ventricular rhythm may be transposed by one induction shock to the normal, which is twice as rapid, when the shock is applied in the diastole. The normal rhythm returns again with the little extrasystole with its brief refractory stage. The normal ventricular rhythm can also return, when toward the end of the ventricular pause an extrasystole is called forth. Then the first sinus impulse incites in the diastole of this extrasystole a short ventricular systole with a brief refractory stage. This short ventricular systole can also induce the normal ventricular rhythm.

5. The normal ventricular rhythm may be transposed to the halved rhythm by calling forth an enlarged ventricular systole. This enlarged ventricular systole is obtained by inciting an extrasystole of the ventricle. Then the postcompensatory systole is broadened and has a lengthened refractory stage. Therefore it can induce the halved rhythm.

6. The ventricular alternation is an intermediate form between the normal and the halved rhythm. The normal ventricular rhythm may be transposed to a ventricular alternation by inciting an enlarged systole. This ventricular alternation may again be transposed to the halved rhythm by calling forth a ventricular systole, which is larger than the large systole of the alternation.

7. A ventricular systole of a definite magnitude can therefore induce a definite ventricular rhythm.

8. The influence of the rapidity of the conduction of the excitation wave on the form of the ventricular electrogram was observed.

9. After artificial transposition of the halved ventricular rhythm to the normal rhythm the contractility and the velocity of the conduction of the excitation wave gradually decrease from the first systole.

10. After transposition of the normal rhythm to the halved rhythm the contractility and the velocity of the conduction of the excitation wave increase from systole to systole.

11. With a definite slowing of the ventricular pulsations, the optimum of the contractility and of the conductivity was reached. These two optima did not coincide.

12. It was established that the mechanic latent stage of the ventricle is lengthened when the velocity of the conduction of the excitation wave through the ventricle diminishes. This happens in a greater measure according as the rate, at which the excitation wave is conducted through the ventricle, is slower.

13. The electric latent stage is also lengthened when the metabolic condition of the ventricle is deteriorated.

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STUDIES ON THE BRAIN STEM

V. Carbon Dioxide Excretion after Destruction of the Optic Thalamus and the Reflex Functions of the Thalamus in Body Temperature Regulation

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In preceding reports (1) attention has been directed to the poikilothermous condition of birds that follows destruction of the optic thalamus. This is not due to temporary shock effects for it persists as long as the animals live. Such animals must be kept at an atmospheric temperature of about 30° C. to continue in good condition, but in this way we have kept them alive for 6 to 10 weeks after operation. The loss of ability to maintain a constant body temperature is not due primarily to a failure of the circulation for it has been found that if the animals are kept in a warm incubator the arterial pressure shows little variation from that of the homothermous decerebrate bird with thalamus intact. This suggested that possibly there might be some depression of the thermogenic mechanism and we have therefore carried out determinations of the carbon dioxide elimination in pigeons rendered poikilothermous by decerebration and cauterization of the optic thalamus. There is of course the uncertainty as to how strictly the carbon dioxide determination alone can be considered an index of heat production but it was assumed that with uniform conditions of diet or starvation it would serve as a indicator of any gross changes.

Methods. A respiratory mask was devised which fitted over the head of the pigeon and the expired air was drawn through sulphuric acid to remove water and the carbon dioxide was absorbed by solid moist lumps of sodium hydroxide. The inspired air was drawn over sodium hydroxide to render it free from carbon dioxide.

The mask was a thin rubber cylinder made from a condum one end of which was slipped over the head and tightly sealed to the neck by 1 per cent collodion, the other end was tied over a T-tube through one

end of which the inspired carbon dioxide free air was drawn and through the other end the expired air passed to the absorption tubes. The pigeon and the entire apparatus except the absorption tubes were set in a constant temperature incubator. Air was drawn through the apparatus by light suction.

Practice soon indicated certain precautions that must be observed with the apparatus.

1. Very thin collodion must be employed, otherwise as it dries it may form a hard constricting ring which may lead to asphyxia or even kill the animals. This is due to occlusion of the neck veins.

2. Light suction must be employed otherwise there may be a similar interference with the venous draining of the head leading to respiration difficulties. This of course would be particularly noticeable with animals in which the skull had been opened as in these decerebrate birds. This effect was avoided in two ways: first, the very thin rubber membrane of which the mask was made, in itself acted as a flutter valve; and second, minimum suction was employed. The only obstructions to the free flow of air through the system were two broad shallow layers of concentrated sulphuric acid through and over which the expired air was drawn. Care was taken that the tubes of sodium hydroxide should not become plugged by deposits of sodium carbonate. That the system was physiologically efficient was indicated by the absence of any respiratory difficulty while the determinations were being made and the fact that they were made repeatedly on birds in which the skull had been opened. Failure to observe any one of these precautions led to difficulties of breathing, as we soon learned.

The pigeon was wrapped in a towel to keep it quiet and the incubator door closed so that the animal was in semi-darkness. By using only adult pigeons, of the common street variety, it was assumed that the body surface area was very near the same and readings are therefore given in units of body weight rather than in terms of surface area.

Results. As in all determinations of normal metabolic rate, the carbon dioxide output of normal adult resting pigeons was found to vary widely with two primary factors: (1) External atmospheric temperature, and (2), state of the digestive activities. The wide variations according to external atmospheric temperature quickly indicated the necessity of keeping the animal at a constant temperature. All readings have therefore been made with the bird in an incubator set at a temperature of 28°–30°C.

Variations according to the state of digestive activity were checked by starvation for at least 3 days before making determinations of carbon dioxide. How important this factor is for birds may be seen by table 1. Thus the average of twenty readings on three normal pigeons on unrestricted mixed diet is 1.88 mgm. of carbon dioxide per hour per gram body weight, in comparison with 1.16 in the starving animals, an increase of 62 per cent. It seems probable that a very great part of this increase is to be attributed to the muscular action of the relatively large muscle stomach or gizzard and part of course may be due to the dynamic action of the absorbed substances. This factor must be taken into consideration since removal of the optic thalamus in the birds nearly always in our experience leads to an immediate disturbance in the digestive tract and hence the carbon dioxide readings on these birds seem abnormally low unless the control animals have been starved for a sufficient time to insure a minimum of activity on the part of the grinding stomach.

TABLE 1

Normal pigeons; birds on unrestricted diet; incubator temperature 21–30°C.

BODY WEIGHT	BODY TEMPERATURE	ROOM TEMPERATURE	NUMBER OF READINGS	CO_2 PER HOUR	CO_2 PER HOUR PER GRAM BODY WEIGHT
	°C.	°C.		mgm.	mgm.
250	41	29	6	444	1.77
270	40–41	27–30	8	485	1.80
266	39–40	27–30	6	548	2.06

With the following precautions taken uniformly as a routine part of the procedure, 1st, as to the use of the respiraion mask; 2nd, preceding starvation for 3 or 4 days; 3rd, atmospheric temperature of 28°–30°C.; 4th, the bird exhibiting no struggles or respiratory difficulties; it was found that the carbon dioxide output of normal birds per gram body weight per hour varied between the extremes of 0.98 and 1.38 mgm. with an average in eleven birds of 1.16 mgm. (table 2).

These are much lower values than those cited by Corin and Van Beneden (2) due to the fact that the atmospheric temperature and feeding conditions were not kept constant by these workers.

In the homothermous pigeon, either the normal bird or the decerebrate bird with cerebral hemispheres removed but thalamus intact, the carbon dioxide elimination varies inversely with atmospheric temperatures below 30°C. (fig. 1). Above 36°C., the carbon dioxide increases as heat polypnea ensues.

The rôle of the feathers in the regulation of body temperature was tested as follows. In two pigeons the feathers were clipped off the entire body so as to leave the skin bare and they were then put in an ice box at 10°–14°C. These animals were left there for 24 hours with readings of body temperature at frequent intervals. The curve of diurnal variations differed only very slightly from that of the normal bird—thus in the normal bird the diurnal variations run on the average from 39° to 41°C. In these birds with feathers removed the curve ran from 38° to 41.5°C. This maintenance of body temperature to almost the normal level for hours when exposed to cold in the absence of feathers, together with the tremendous increase in carbon dioxide excretion in

TABLE 2

Normal pigeons; 3 to 5 days starvation; body temperature 39–40°C.; incubator temperature 30°C.

BODY WEIGHT	NUMBER OF READINGS	CO_2 PER HOUR	CO_2 PER HOUR PER GRAM BODY WEIGHT
	°C.	*mgm.*	*mgm.*
258	2	236	0.91
260	2	360	1.38
245	2	246	1.00
267	2	298	1.12
223	2	288	1.29
266	2	364	1.36
258	2	322	1.24
259	4	307	1.18
265	2	344	1.29
256	2	252	0.98
241	7	298	1.23

the bird with feathers intact when exposed to cold, indicate that in birds the primary factor in maintenance of body temperature against cold lies in increased heat production and protection against heat loss is a secondary factor.

Removal of the cerebral hemispheres with the thalamus left intact does not reduce the carbon dioxide output. Thus in a pigeon under standard conditions of temperature and starvation, from which the hemispheres were removed and a functional thalamus was indicated by *a*, the fluffed position of the feathers; *b*, decerebrate restlessness and *c*, a normal body temperature, the average of three determinations was 1.10 mgm. of carbon dioxide per gram body weight per hour in comparison with the average of normal birds of 1.16 mgm. Further-

more such a decerebrate bird responds to exposure to a cold atmosphere by an increased carbon dioxide production and to heat (35°C.) by "panting" (fig. 1). This confirms the findings of Corin and Van Beneden (2) that removal of the cerebral hemispheres alone does not alter the thermogenic ability of the bird or its ability to regulate its body temperature through wide variations of the atmospheric temperature.

The carbon dioxide output of seven poikilothermous pigeons was determined with the following precautions:

1. To avoid possible depression of metabolism due to the cerebral traumatism or to shock, readings have been made at time intervals of 2 to 40 days after the operation.

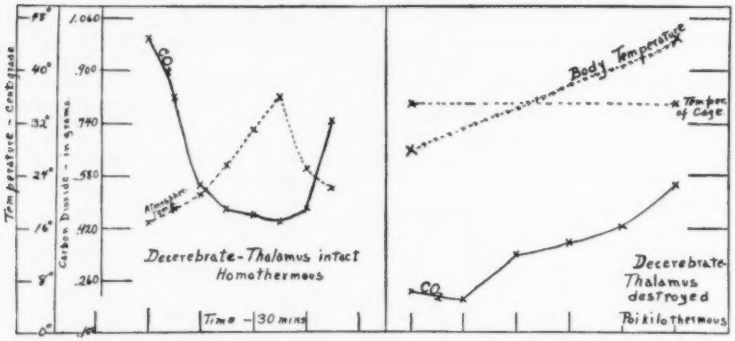

Fig. 1. Variations in carbon dioxide elimination in homothermous and poikilothermous decerebrate pigeons with variations of atmospheric temperature and body temperature. *a*) Curves to left, the homothermous pigeon. *b*) Curves to right, the poikilothermous pigeon.

2. The birds were starved from 2 to 4 days before the readings were made.

3. The attempt was made to keep the animal at an atmospheric temperature of 30°C., so as to keep the body temperature close to the normal value of 40°–41°C. It is not easy without constant slight changes of incubator temperature to keep the operated birds' temperatures within these limits.

4. The customary routine precautions in the use of the respiratory mask.

With these precautions the readings given in table 3 were obtained. It will be noted that in the pigeon in which the optic thalamus has been destroyed, if the body temperature is kept normal by being kept in a

warm atmosphere, the carbon dioxide elimination is very nearly the same as in the normal pigeon. As the body temperature is allowed to fall below 40°C., the carbon dioxide output also declines and varies directly with the body temperature between the wide limits of 27° to 44°C. (fig. 1). In this respect the results are analogous to the readings in cold-blooded animals (3). Freund and Grafe (4) find the same to be true of rabbits rendered poikilothermous by transection of the cervical cord. If the carbon dioxide output parallels the heat production of the body, these readings evidently indicate that normal heat production—thermogenesis—in the pigeon is not necessarily dependent on the

TABLE 3

Birds with optic thalamus destroyed; 2 to 4 days starvation; incubator temperature 27–30°C.

BODY WEIGHT	BODY TEMPERATURE	DAYS AFTER OPERATION	NUMBER OF READINGS	CO_2 PER HOUR	CO_2 PER HOUR PER GRAM BODY WEIGHT
	°C.			mgm.	mgm.
220	38.0	4	2	228	1.03
235	39–40	4	4	296	1.25
211	39.0	2	2	342	1.62
215	40.0	5	2	242	1.12
284	38.0	4	3	244	0.85
226	41.0	2	2	219	0.96
232	38.0	3	2	203	0.87
250	37.5	48	2	249	1.00
251	27.0	5	2	219	0.87
220	36.0	4	2	196	0.89

temperature regulating centers of the thalamus but in this case is determined by the temperature of the tissues themselves, howsoever the tissue temperature be determined. At this point it should be emphasized that we are here dealing with lesions of the thalamus and not of the midbrain. Midbrain injuries in birds lead to violent muscular disturbances which have been rigidly excluded in this series. The absence of any appreciable midbrain injury is indicated by the complete absence of any visible disturbances of muscular coördination and the longer period of time that the bird with thalamic injury only may be kept living.

DISCUSSION

From the preceding we draw the conclusion that whatever may be the rôle of the thalamus in body temperature regulation, the quantity of heat production in the tissues is not of necessity dependent on this nerve center but is a function of the temperature of the tissues, howsoever that be fixed.

After removal of the optic thalamus in a pigeon, it differs from one with the thalamus intact in this respect: although the elimination of carbon dioxide is the same in both cases provided the body temperature in both is identical, the latter responds to atmospheric cold by an increase in carbon dioxide, the former by a decrease which varies directly with the extent of the atmospheric change.

Reviewing the preceding studies of this series we believe the following statement can be made as to the mode of action of the thalamus in regulating body temperature.

Thermogenesis is a function of the tissues not primarily dependent on this center but determined by conditions in the tissues themselves. Increased heat production when exposed to atmospheric cold is one of the primary essentials for the homothermous condition in the bird with protection against heat loss being a secondary factor. Although a minor factor, conservation of body heat against loss by radiation from the skin is undoubtedly effected to some extent by the feathers. The feathers are movable having a double set of muscles by which they may be either depressed or elevated. These muscles are innervated by sympathetic nerves (5). In the quiet normal bird exposed to cold the feathers are characteristically fluffed. Apparently this prevents or reduces contact of the cold with the skin, and reduces radiation from the skin although, so far as we know, no measurements on this point have been made. This elevation of the feathers to its full extent as seen in the sleeping bird or in the typical decerebrate bird is *dependent* on a functional thalamus. The change in position of the feathers after decerebration is an old observation noticed by earlier workers on the bird brain (Flourens, Schader, Munk). Becheterew (6) seems to have been the first to note specifically that the fluffed or depressed position of the feathers is determined by whether or not the thalamus is functional after decerebration. After destruction of the thalamus slight changes in feather erection occur dependent on body temperature variations (7) but never the complete elevation that is to be seen so characteristically either in the normal sleeping bird or in the bird with hemispheres only removed.

Heat dissipation in the homothermous pigeon is principally by evaporation or radiation from the lungs. When exposed to temperatures of increasing warmth there is a progressive reduction in heat production and at about 36°C., there is the onset of heat polypnea insuring a greater evaporation.

After destruction of the thalamus the following factors of regulation disappear:

1. Depression of the feathers against the body with resulting greater exposure of the skin to either cold or warmth.

2. Disappearance of polypnea when exposed to higher atmospheric temperatures and resulting hyperpyrexia of the animal.

3. Heat production in the tissues determined according to local tissue temperatures and not *inversely* by atmospheric temperatures.

4. Blood pressure varying directly with the body temperature and not constant independently of atmospheric temperature.

On the basis of these facts we advance the following view as a working hypothesis for further studies on the reflex functions of the thalamus.

Reflex augmentation of muscle tone or activity leading to increased heat production from stimulation of sensory nerve endings of cold is dependent on an intact thalamus. Heat polypnea or "panting" when exposed to excessive warmth is dependent not on the medullary centers alone but involves the thalamus. This may be either a reflex effect on the nerve endings or possibly one involving blood temperature. That the medullary centers alone are unable to carry out the muscular coördination required in "panting" is indicated by the work of various men on respiratory centers above the medulla oblongata as well as by the facts we report. Thus Nicolaides and Dontas (12) state that heat polypnea cannot be produced if the corpora striata be separated from the medulla. Such work will require a study of the neuro-muscular mechanism of "panting" in contradistinction to mere increase in rate or amplitude of breathing or hyperpnea. In birds reflex changes in the position of the feathers according to atmospheric temperature, namely, fluffing when exposed to cold and depression when exposed to heat, requires thalamic activity.

It is a well-known fact that painful stimuli (or noci-ceptive stimuli of Sherrington) induce reflex changes in striated muscle tone after separation of the spinal cord from the brain. Our results suggest that this is probably not true of milder stimulation of the cold and warmth nerve endings of the skin, but that sympathetic and muscle tone *reflexes*

from stimulation of the cutaneous temperature receptors, involve the thalamus as an essential part of the functional pathway. Or in the terms of Sherrington, reflex correlation between exteroceptive receptors of the skin (not including pain) and proprioceptive and interoceptive receptors, requires a higher complexity of integration than do the simple spinal reflexes of skeletal muscles induced by painful stimuli.

The only experimental work directly related to this view is that of Martin, Franklin and Hield and of Barbour. Barbour (8) finds that transection of the spinal cord in dogs abolishes the reaction of shivering when exposed to moderate depression of atmospheric temperatures. Martin and his co-workers (9) have studied vasomotor reflexes induced by warmth and cold applied to the skin of decerebrate rabbits. Unfortunately their work does not furnish crucial evidence either for or against the proposed view of thalamic functions since they did not differentiate between decerebrations with or without removal of the thalamus. But the view we propose may be the anatomical counterpart of their conclusion as to the vasomotor response being determined by the "quantity of nervous discharge" rather than to "stimulation of specific sense organs." The relatively large masses of grey matter constituting the thalamus, we suggest, are involved in the determinations of this "quantity of discharge" to which they refer. It is needless to add that this does not exclude a similar, possibly even a greater, action from the cerebrum itself.

We do not over-emphasize the importance of reflexes from temperature nerves in body temperature regulation for it seems to us that direct changes in the brain centers brought about by changes in blood temperature are probably more important as is emphasized in Barbour's work (10). Furthermore Ischensmid and Krehl (11) who, unknown to us until after this paper was written, have practically duplicated our work, using rabbits instead of pigeons, attempt to localize the centers of the thalamus necesary to body temperature regulation in the relatively small posterior, ventral and central grey regions.

SUMMARY

Removal of the cerebral hemispheres of pigeons, leaving optic thalamus intact, does not appreciably alter the output of expired carbon dioxide in resting starving birds, nor does it alter their ability to regulate body temperature against atmospheric cold by increased heat formation and against warmth, by polypnea.

In pigeons rendered poikilothermous by destruction of the optic thalamus, the carbon dioxide output varies directly with the body temperature variations. If the body temperature is set to a normal level by regulation of the atmospheric temperature, the output of carbon dioxide falls within the limits of variations of normal homothermous birds. After removal of the thalamus the pigeon does not respond to atmospheric cold by increased heat production nor to warmth (36°C.) by "panting" (polypnea).

It is suggested that reflex changes of skeletal muscle tone and of the sympathetic system induced by stimulation of the temperature nerves of the skin involve the thalamus as an esssential part of the functional pathway.

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STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

IX. On the Relation of Stale Sperm to Fertility and Sex in Ring-Doves

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The abnormal sex ratios observed in hybrid birds by Suchetet (1), Guyer (2), Whitman (3), Riddle (4), (5), (6), Smith and Haig-Thomas (7) and Phillips (8) justify any inquiry which may be made on either the spermatozoa or ova of these forms if the result assists in finding a factor, or in excluding what may by some investigators be considered a factor, in the causation of such abnormal sex ratios.

"Staleness" or "over-ripeness" of ova was one of the conditions associated with the very abnormal sex ratios which Hertwig (9) and Kuschakewitsch (10) obtained in experiments on sex determination in the frog. There is probably no good evidence that staleness of sperm is anywhere associated with abnormal sex ratios. In the extensive studies on sex in doves and pigeons being conducted by one of us it is desirable, however, to know whether stale germ cells are a complicating factor in the ratios obtained. The possibility of very stale ova of the bird being fertilized is excluded; but the possibility of the egg being fertilized by stale sperm certainly exists. It seems perhaps improbable that staleness of the sperm would influence sex in this material since in birds the differentiation of the germ cells with respect to sex almost certainly resides in the ova, not in the sperm; other and probably better reasons might also be stated. Nevertheless, the subject requires the test of experiment.

Moreover, the length of time during which spermatozoa may retain their fertilizing power in the oviduct of the female dove has apparently remained quite unknown. In breeding work it is sometimes necessary to know the duration of this period. The present work was so conducted that, whatever the result concerning the relation of stale sperm

to the sex ratio, we should definitely learn the age at which the stale spermatozoa of the dove lose their power to fertilize.

MATERIALS AND METHODS: The females used were nearly all hybrids of two closely related species of ring-doves—*Streptopelia risoria* and *St. alba*. Females 49 and B442 were hybrids involving the two last-named species and the Japanese ring-dove (*St. douraca*). At the beginning of these studies the thirteen females successfully studied were of ages varying from about 18 months to 5 years. Nine of the males were hybrids of the same kind as the females first mentioned above. Two (tables 1 and 3) were hybrids of the three above-named species. One pure-bred blond ring (*St. risoria*) was used (table 6). Normally, all of the above described pairs are fully fertile. In one case (table 11) a generic hybrid male was used which, so far as known, is not fully fertile in any cross.

Infertile eggs were occasionally produced by some of these birds before the experiment began. Most of the females used had been made to lay eggs with abnormal frequency. During the experiment a few eggs were obtained whose infertility is obviously due to the same factors which caused it prior to the beginning of the experiment.

Each pair was confined in a separate pen. The males were allowed their usual complete freedom of the pen with the female during (usually) 1, 2 or 3 days after the laying of a pair of eggs. In the latter part of the study this period was often shortened to 1 day or less. Thus in a few cases the separation was made after only 2 or 3 hours; for the shortest of such periods the birds were watched until one or more copulations had occurred. The male was then confined in a smaller cage placed within the pen belonging to the pair.[1] The date and hour of confinement of the male was noted. The time of "separation of the parents," as this is indicated in tables 1 to 11, has been reckoned from the hour of separation to the hour of the laying of the next following egg. It is practically certain that fertilization occurs, if at all, very soon after the egg leaves the ovary. Since in these doves ovulation occurs 40 to 44 hours[2] before the egg is laid it is clear that 1 day or slightly more should be deducted from the figures for staleness of

[1] The fact that female ring-doves require stimulation of a sexual nature for the production of mature eggs makes it advisable or necessary to leave the male in plain sight and as nearly in contact with the female as is possible and still prevent copulation. The period during which the birds were left together after the laying of a pair of eggs was intended to insure the beginning of growth in the next pair of ovarian eggs.

[2] Riddle, unpublished data.

sperm as given in the tables. On the other hand, in most instances it is not certain that one of the last cells liberated by the male is the one that fertilizes the egg. On the basis of chance it probably is usually a somewhat older sperm that accomplishes the fertilization, except in those cases in which all such older sperms have completely lost their power to fertilize or for which sperms from only a single copulation were available.

It sometimes happened that a female failed to produce eggs after the removal of the male; and a few females failed to lay eggs under these conditions in all of the trials made with them. These latter complete failures have not been tabulated nor otherwise considered. In other occasional failures—when eggs were not produced after a reasonable period of separation—the pair was reunited until eggs were again laid and the results of such fertilizations with normal sperm are given in parentheses in columns 3 and 6 of tables 1 to 11, and are summarized in a section of tables 12 and 13.

All of the eggs studied were incubated by pairs of birds kept for such a purpose. Fertility was doubtless somewhat reduced in four pairs of birds by the tuberculosis which developed in one or both parents during the course of the year utilized for this investigation.

PRESENTATION OF DATA: *Stale sperm and fertility.* The records of each of the several pairs may first be examined with a view to learning the length of the period which sperm cells may remain in the female genital tract and retain their fertilizing power. The data indicate that this period was nearly the same for several of the pairs.

In table 1 it will be observed that all except 7 of the 63 eggs tested were fertile, and that for 6 of the 7 exceptions only very stale sperm was available for fertilization—6.2, 6.7, 7.7 (two cases), 7.9 and 8.7 days. In one case an egg was infertile although normal sperm was presumably available. Nine eggs with 7.7-day periods were fully fertile.

The pair whose record is given in table 2 was somewhat less fertile than the preceding pair. This female often refused to produce eggs when separated from the male. For this reason 16 eggs shown in the table were fertilized by normal sperm. Two of this group of eggs showed only slight development. Of the 46 eggs fertilized by stale sperm 16 were wholly infertile (3 others produced 1-day embryos or less). Nine of the 16 infertile eggs were associated with sperm of 7 to 13.7 days of staleness. One egg was fertilized 8 days after separation of the male. Three eggs produced at 9.7 to 18.7 days after separation of the male were wholly infertile. The fertilizing power of the stale

TABLES 1 AND 2

Effects of fertilization with stale sperm in females E361 (left) and 49 (right)

TABLE 1

Date of eggs	Days of separation of parents	Fertility and sex; remarks	Date of eggs	Days of separation of parents	Fertility and sex; remarks
11/25	(0)	(♀)	4/19	7.0	♀
11/27	(0)	(♂)	4/21	8.7	0*
12/4	5.0	♀	4/27	6.0	♂
12/6	6.7	♂	4/29	7.7	♂
12/13	5.0	♀	5/5	6.0	2-day
12/15	6.7	♀	5/7	7.7	0
12/22	3.3	?♀	5/13	6.0	♂
12/24	5.0	♂	5/15	7.7	♀
12/31	5.0	♂	5/21	6 0	♀
1/2	6.7	♀	5/23	7.7	♀
1/8	4.0	♀	5/29	6.0	♀
1/10	5.7	♂	5/31	7.7	♀
1/17	5.0	♀	6/6	6.0	6-day[3]
1/19	6.7	♂	6/8	7.7	3-day[3]
1/26	5.0	♂	6/14	6.2	0
1/28	6.7	♂	6/16	7.9	0
2/3	4.0	♂	6/21	4.3	♂
2/5	5.7	♀	6/29	6.0	♀
2/11	4.3	♂	7/1	7.7	♂
2/13	6.0	♂	7/6	4.3	4-day
2/19	4.0	♂	7/8	6.0	4-day
2/21	5.7	♂	7/14	6.0	♂
2/28	6.0	♀	7/16	7.7	♀
3/2	7.7	♂	7/22	6.0	♀
3/9	6.0	♂	7/24	7.7	Sl.[2]
3/11	7.7	0*	8/14	(0)	(♂)
3/17	5.0	♀	8/16	(0)	(♂)
3/19	6.7	♂	8/22	6.0	♂
3/25	5.0	♂	8/24	7.7	♀
3/27	6.7	0	9/14	(0)	(0)
4/2	5.0	♀	9/16	(0)	(♂)
4/4	6.7	♂			
4/10	5.0	♀			
4/12	6.7	♂			

TABLE 2

Date of eggs	Days of separation of parents	Fertility and sex; remarks	Date of eggs	Days of separation of parents	Fertility and sex; remarks
8/21	3.0	0*	4/9	6.0	♀
8/23	4.7	0	4/11	7.7	0
8/29	4.0	♂	4/20	8.0	Fert.[1]
8/31	5.7	1-day	4/22	9.7	0
9/6	4.0	Br.[1]	4/29	6.0	0
9/8	5.7	♀	5/1	7.7	0
9/15	5.0	♀	5/8	6.0	Sl[2]
9/17	6.7	♀	5/10	7.7	0
9/23	4.0	♀	5/19	8.0	0
9/25	5.7	♂	5/21	9.7	Br.[1]
10/1	4.0	♂	5/28	5.3	♀
10/3	5.7	♀	5/30	7.0	0
10/10	5.0	♀	6/5	4.0	♀
10/12	6.7	Br.[1]	6/7	5.7	♀
11/2	(0)	(Hat.)[4]	6/13	5.2	0
11/4	(0)	(Sl.)[2]	6/15	6.9	0
11/23	(0)	(Sl.)[2]	6/22	5.0	♂
11/25	(0)	(♀)	6/24	6.7	0
12/18	(0)	(♂)	7/2	7.0	♀
12/20	(0)	(♂)	7/4	8.7	Dis.[5]
1/8	(0)	(♀)	7/10	4.0	♀
1/10	(0)	(?♀)	7/12	5.7	♀
1/18	6.0	11-day	7/20	6.0	♀
1/20	7.7	0	7/22	7.7	Sl.[2]
2/8	(0)	(♀)	7/30	6.0	0
2/10	(0)	(♀)	8/1	7.7	Dis.[5]
3/1	(0)	(♂)	8/9	6.0	♀
3/3	(0)	(♀)	8/24	2.2	♀
3/10	5.0	♂	8/26	3.9	♀
3/12	6.7	4-day	9/7	12.0	0
3/19	5.0	?♀	9/9	13.7	0
3/21	6.7	13-day	9/16	(0)	(?♂)
3/31	(0)	(♀)	9/18	(0)	(♀)
4/2	(0)	(♀)			

*Signifies "not fertile."

[1] Egg was broken.

[2] Egg showed slight development

[3] Embryo killed by accident.

[4] Bird hatched; died, decayed before examination.

[5] Egg disappeared (almost certainly broken in nest).

TABLES 3 AND 4

Effects of fertilization with stale sperm in females B442 (top) and A684

DATE OF EGGS	DAYS OF SEPARATION OF PARENTS	FERTILITY AND SEX; REMARKS	DATE OF EGGS	DAYS OF SEPARATION OF PARENTS	FERTILITY AND SEX; REMARKS
1/27	(0)	(♂)	5/27	4.3	♀
1/29	(0)	(♂)	5/29	6.0	♀
2/4	4.0	♀	6/4	5.0	Some development
2/6	5.6	♀	6/6	6.7	♀
2/12	4.0	♂	6/13	6.0	♀
2/14	5.7	♂	6/15	7.7	2-day embr.; killed
2/20	4.0	♀	6/23	6.3	♂
2/22	5.7	♂	6/25	8.0	0*
2/28	4.0	♂	7/1	4.4	♀
3/2	5.7	♂	7/3	6.1	♀
3/8	4.0	♂	7/9	4.0	♀
3/10	5.7	♂	7/11	5.7	Egg broken
3/16	4.0	♀; 13-day embryo	7/17	4.0	?♂; 10-day embryo
3/18	5.7	Egg disappeared	7/19	5.7	Fertile; broken
3/24	4.0	♀	7/25	5.0	♂
3/26	5.7	♂	7/27	6.7	♀
4/1	5.0	♀	8/2	4.0	11-day embryo
4/3	6.7	♂	8/4	5.7	7-day embryo
4/9	5.0	♂; 13-day embryo	8/10	4.0	5-day embryo
4/11	6.7	♂; 13-day embryo	8/12	5.7	♂
4/17	5.0	♂	8/18	4.0	5-day embryo
4/19	6.7	♀	8/20	5.7	5-day embryo
4/25	5.0	♂; 12-day embryo	8/26	6.2	4-day embryo
4/27	6.7	5-day embryo	8/28	7.9	3-day embryo
5/3	4.3	♀	9/4	7.1	8-day embryo
5/5	6.0	6-day embryo	9/6	8.8	0
5/11	5.0	♂	9/13	(0)	4-day embryo
5/13	6.7	Egg disappeared	9/15	(0)	3-day embryo
5/19	5.0	Deformed (decayed)	9/22	(0)	(♂)
5/21	6.7	♀	9/24	(0)	(8-day embryo)[1]
3/15	8.0	0*	6/8	6.0	♀
3/17	9.7	0	6/10	7.7	0
4/3	5.2	♀	6/16	5.0	♀
4/5	6.9	♂	6/18	6.7	♂
4/11	4.0	♂	6/25	6.0	♂
4/13	5.7	♀	6/27	7.7	♀
4/19	4.0	♀	7/4	6.0	♀
4/21	5.7	♂	7/23	(16 or 0)[2]	(♂)
4/27	4.0	♀	7/25	(0)	(♂)
4/29	5.7	♀	8/1	5.3	♀

TABLES 3 AND 4—*Concluded*

DATE OF EGGS	DAYS OF SEPARATION OF PARENTS	FERTILITY AND SEX; REMARKS	DATE OF EGGS	DAYS OF SEPARATION OF PARENTS	FERTILITY AND SEX; REMARKS
5/6	6.0	♀	8/3	7.0	♂
5/8	7.7	♀	8/9	4.0	♀
5/21	12.0	0	8/11	5.7	♀
5/23	13.7	0	9/13	(0)	(♂)
5/30	5.0	♀	9/15	(0)	(♀)
6/1	6.7	♂; 12-day embryo			

* Signifies "not fertile."

[1] Probably most of the eggs laid at this period had shells that were too thin. The early death of these embryos was probably associated with these defective shells.

[2] The male was separated from the female 16 days before this egg was laid. He was again admitted 31 to 32 hours before the egg was laid. Normally the egg requires more than this amount of time to descend the oviduct; it is also normally fertilized soon after entering the oviduct. It seems extremely probable, however, that this egg was fertilized by a fresh, not by a stale, sperm.

sperm cells of this pair was retained probably between 8 and 9 days. In only one other instance (table 8) in this study was an egg fertilized when the period of separation from the male was as much as 8 days.

Table 3 shows the result of testing 51 eggs with fertilization by stale sperm. The degree of staleness varied between 4 and 8.8 days. All eggs were fertile except the two which were laid after longest separation of the male, 8 and 8.8 days. One egg with a 7.9-day period was fertile. Many of the embryos of this record died early. This will also be noted in the records of three additional females which were used in the present study. These several records may properly raise the question whether fertilization by stale sperm is connected with the short life-term of the embryos. Data obtained from the other birds used in this study indicates, however, that no such connection exists; and a later special examination of the eggs produced by these birds has shown that not stale spermatozoa but inadequate egg-shells and some further deficiency or weakness of the ovum itself are associated with the early death of these embryos (16). No evidence was obtained in any part of this work which would indicate weakness or modified viability due to stale sperm parentage; the hatched young were, however, killed at an early age.

Table 4 gives the results of 27 tests with stale sperm. All eggs were fertile except 4 in which the degree of staleness exceeded 8 days (8 to

TABLES 5 TO 7

Effects of fertilization with stale sperm in females 79 (top), 744 (middle), and 192

DATE OF EGGS	DAYS OF SEPARATION OF PARENTS	FERTILITY AND SEX; REMARKS	DATE OF EGGS	DAYS OF SEPARATION OF PARENTS	FERTILITY AND SEX; REMARKS
3/17	(0 or 14)	(4-day embryo)	6/11	5.0	♂
3/19	(0)	(♀; 12-day embr.; killed)	6/13	6.7	♀
3/26	5.0	♂	6/19	5.0	Some (abnor.) devel.
3/28	6.7	♂	6/21	6.7	Egg broken
4/3	4.0	♀	6/27	5.0	♂
4/5	5.7	♀	6/29	6.7	0*
4/11	4.0	♀	7/6	6.0	♂
4/13	5.7	♀	7/8	7.7	0
4/20	5.0	♂	7/14	5.0	Abnor. vasc. area
4/22	6.7	♀	7/16	6.7	♀
4/29	5.0	4-day embryo	7/23	6.0	♂
5/8	6.0	Egg broken	7/25	7.7	0
5/10	7.7	4-day embryo	8/1	5.3	0
5/16	5.0	♀	8/3	7.0	0
5/18	6.7	*No gonads*; 14-day embr.	8/9	4.0	♂
5/24	5.0	Egg disappeared	8/11	5.7	♀
5/26	6.7	♀	9/13	(0)	(?♂)
6/2	6.0	♀	9/15	(0)	(♂)
6/4	7.7	♀			
7/4	4.0	Fertile; egg broken	8/13	4.0	♂
7/6	5.7	♂	8/15	5.7	♂
7/12	4.0	Slight (abnor.) devel.	8/21	5.0	Good vasc. area
7/14	5.7	♂	8/23	6.7	♂
7/20	4.0	Egg broken	8/29	6.2	Egg broken
7/22	5.7	♀; 11-day embryo	8/31	7.9	9-day embr.; killed
7/28	4.0	Some development	9/6	6.2	5-day embryo
7/30	5.7	♂	9/8	7.9	3-day embryo
8/5	4.0	Slight (abnor.) devel.	9/14	(0)	2-day embryo
8/7	5.7	2-day embryo; abnor. (?)	9/16	(0)	(♂)
8/15	5.0	0*	2/10	4.0	♀
8/24	5.0	0	2/12	5.7	0
9/1	4.0	♀	2/18	4.0	♂
9/11	6.0	0	3/26	(0)	(2-day embryo)
9/19	4.0	0	3/28	(0)	(3-day embryo)
9/21	5.7	?♀; decayed	4/3	4.0	5-day embryo
9/27	4.0	Slight development	4/5	5.7	♀
9/29	5.7	♀	4/10	4.0	♀
10/5	4.0	♀	4/12	5.7	♀
10/30	(0)	(Slight development)	4/18	5.0	♀

TABLES 5 TO 7—*Concluded*

DATE OF EGGS	DAYS OF SEPARATION OF PARENTS	FERTILITY AND SEX; REMARKS	DATE OF EGGS	DAYS OF SEPARATION OF PARENTS	FERTILITY AND SEX; REMARKS
11/1	(0)	(♂)	4/20	6.7	9-day embryo
11/8	5.0	4 day embryo	4/26	5.0	♀
11/17	5.0	♀	4/28	6.7	Egg broken
11/26	5.0	♂	5/4	5.0	0
12/19	(0)	(0)	5/6	6.7	0
12/21	(0)	(♀)	5/12	5.0	♂
12/28	5.0	♀	5/14	6.7	♂
1/6	5.0	0	5/20	5.0	♀
1/8	6.7	0	5/22	6.7	♀
1/15	5.0	♀	5/29	6.0	♂
1/24	5.0	0	5/31	7.7	♂
1/26	6.7	0	6/6	5.0	0
2/2	5.0	0	6/8	6.7	0
			6/14	5.0	♂
			6/16	6.7	♂

*Signifies "not fertile."

13.7 days) and one other in which this period was 7.7 days. In two cases eggs were successfully fertilized 7.7 days after separation of the male.

The pair recorded in table 5 produced 34 eggs which were tested for fertility. Four eggs fertilized by normal sperm were all fertile. Also, all of the 11 eggs fertilized by sperm of 5 days or less of staleness were fertile. Four eggs, with 5.3 to 7-day periods were infertile. Two of the four tests made of a 7.7-day period were infertile.

The record given in table 6 was considerably influenced by the fact that most of the eggs laid were provided with inadequate shells. This and probably some other deficiency of the eggs doubtless prevented many of the embryos from completing their development. All of these eggs were fertile, and two of them were fertilized by very stale sperm (7.9 days). The male used in this instance was a pure-bred blond ring-dove. Since this was the only pure-bred successfully used in this study, and since the longest period tested was only 7.9 days and this proved fertile, we have no data on the question as to whether the sperm of pure-breds retains its fertility for a longer period than does the sperm of hybrids. This single instance affords some evidence that the fertility is not retained for a shorter time in the non-hybrid male.

TABLES 8 TO 10

Effects of fertilization with stale sperm in females B629 (top), A91 (center), and miscellaneous (below)

DATE OF EGGS	DAYS OF SEPARATION OF PARENTS	FERTILITY AND SEX; REMARKS	DATE OF EGGS	DAYS OF SEPARATION OF PARENTS	FERTILITY AND SEX; REMARKS
9/3	8.0	Some development	5/13	4.0	♂
10/19	(0)	(♀)	5/15	5.7	Soft-shelled egg
10/21	(0)	(6-day embryo)	5/21	4.0	5-day embryo
11/7	11.0	0*	5/23	5.7	5-day embryo
11/9	‡2.7	0	5/29	4.0	♂
12/11	(0)	(Some development?)	5/31	5.7	4-day embryo
12/20	4.3	Egg broken	6/6	4.0	6-day embryo
1/16	(0)	(?♀)	6/8	5.7	Egg disappeared
1/18	(0)	(3-day embryo, killed)	6/14	4.0	0
2/4	(0)	(7-day embryo)	6/16	5.7	0
2/6	(0)	(11-day embryo)[1]	6/22	4.3	0
2/15	7.0	0	6/30	5.0	0
2/24	5.0	0	7/2	6.7	0
2/26	6.7	Some devel.; broken	7/8	5.0	♀
3/5	5.0	Egg broken	7/10	6.7	0
3/14	4.0	♀	7/16	4.3	0
3/16	5.7	0	7/24	5.0	Slight development
3/23	4.1	0	7/26	6.7	0
3/31	4.0	♂	8/1	5.0	4-day embr.; killed
4/2	5.7	6-day embryo	8/3	6.7	Egg broken
4/8	4.0	4-day embryo	8/9	5.0	♂
4/10	5.7	5-day embryo	8/11	6.7	0
4/17	5.0	5-day embryo	8/17	5.0	Slight development[2]
4/25	4.0	♀	8/31	(0)	(Thin shell, broken)
4/27	5.7	3-day embryo; killed	9/2	(0)	(Soft-shelled egg)
5/5	6.0	Some devel.; broken	9/13	(0)	(♀)
5/7	7.7	Egg disappeared	9/24	(0)	(♀)
3/12	5.0	♂	4/22	4.0	3-day embr.; killed
3/14	6.7	♀	4/24	5.7	♀
3/21	5.0	Some development	4/30	5.0	7-day embryo[3]
3/23	6.7	♀	5/2	6.7	6-day embryo[3]
3/29	4.0	Trace of development	5/11	8.0	Egg disappeared
3/31	5.7	?♀; gonads atypical	5/13	9.7	Egg disappeared
4/6	3.2	0*	5/20	6.0	♀
4/8	4.9	9-day embryo	5/22	7.7	♀
4/14	5.0	♀	5/31	8.0	0
4/16	6.7	♂	6/2	9.7	0[4]

TABLES 8 TO 10—*Concluded*

♀ E562			♀ K363			♀ A60[5]		
10/27	12.0	0*	7/8	12.3	0	8/20	5.0	♀
1/8	15.0	0	7/10	14.0	0	8/22	6.7	♀
1/10	16.7	0	7/31	(0)	(♂)	10/10	(0)	(♂)
1/18	6.0	♀	8/2	(0)	(♂)	10/12	(0)	(♀)

* Signifies "not fertile."

[1] In this table, as in tables 3 and 6, it is probable that the early death of many of the embryos was causally associated with the abnormally thin shells of the eggs and with other deficiency within the egg.

[2] Male not copulating later; diseased, killed 8/24. Tubercular and with atrophied testes. Another male given 8/25.

[3] Death resulted from poor incubation.

[4] Female parent (A91) dead (internal hemorrhage, some tuberculosis) 28 days after.

[5] These three females refused in other trials to produce eggs when their mates were isolated.

The records for female 192 (table 7) and for the three additional females next to be considered are somewhat complicated by the fact that one or both of the parents became tubercular during the progress of the experiment. In these cases it is probable that part of the infertility found may be more properly assigned to disease in the parent than to the staleness of the sperm. The tests recorded in table 7 show much infertility, but this infertility is also shown in one of four tests with normal sperm. Further, the longer terms of staleness are not clearly associated with a higher percentage of infertility than are the shorter terms of staleness. The longest period tested was 7.7-days and this showed full fertility. Autopsy of the male at the close of the period studied showed tuberculosis and partial atrophy of the testes.

The tests recorded in table 8 are much like those found in table 7. In the present case, however, the absolute infertility of the two longest periods of staleness, 11 and 12.7 days, is probably significant. An 8-day period tested fertile although four of the five 6.7-day periods tested were wholly infertile. It will be noted that most of the fertile eggs of this record, like those of tables 3 and 5, produced embryos which died early.

Table 9 gives the result of 20 tests on fertility. All were fertile except the two tests made of the stalest sperm; the periods testing infertile were of 8 and 9.7 days. One test at 7.7 days and four at 6.7 days were fertile.

In table 10 is recorded the small amount of data obtained from three different females, (E562, K363, A60). Female E562 tested fertile in one case with sperm 6 days old, and three times infertile with sperm 12, 15 and 16.7 days old. Here the range of fertility apparently lies between 6 and 12 days. No. K363 was fully fertile in normal matings but was not fertile after a separation of the male for 12.3 and 14 days. No. A60 gave two fertile tests at 5 and 6.7 days. This female laid some soft-shelled eggs and others with shells which were probably too thin. Several of these eggs although of normal appearance, were fertile when fertilized by normal sperm but, like those already noted in tables 3, 5 and 8, most of the embryos died before hatching. All of the three females of this table failed to produce eggs when separated from the male except in the instances tabulated.

The record for fertility presented in table 11 is complicated not only by the fact that both parents were tubercular at the close of the experimental period, but by the additional circumstance that the male parent was a generic hybrid. Therefore, wholly apart from the matter of staleness of sperm and of the tuberculosis which developed in the parents, successful fertilization of all eggs could not be expected. Nevertheless the data seem to indicate that the staler sperm (5.7 and 6.7 days) are more frequently associated with infertility. None of the eggs laid longer than 5.7 days after separation of the parents was fertile although five such eggs were tested. Fertility in this pair of birds probably did not continue beyond 6 or 7 days. A notable summary is added to this table.

The summary on fertilization with normal and with stale sperm given in table 12 makes it clear that the stale sperm are associated with greater infertility than are the normal sperm. Also, the longer periods of staleness ("5 days," and "6+ days") show progressively greater infertility.

Stale sperm and the sex ratio. Whether in these experiments staleness of sperm affects the sex ratio may be next considered. The summarized data of table 12 can be first used to test this point. In this table only absolute infertility and embryos or offspring whose sex was learned are recorded. Embryos which died before their sex could be learned are left out of account. An adequate interpretation of the sex data requires a full and rather long discussion.

It will first be noted that we have not obtained a large number of male and female offspring from the "stale sperm fertilizations." The total number of such offspring is 213. Three divisions are made,

TABLE 11

Effects of fertilization with stale sperm in female A417

DATE OF EGGS	DAYS OF SEPARATION OF PARENTS	FERTILITY AND SEX; REMARKS	DATE OF EGGS	DAYS OF SEPARATION OF PARENTS	FERTILITY AND SEX; REMARKS
8/29	5.0	♂	4/7	4.0	♂
9/6	4.0	0*	4/9	5.7	0
9/8	5.7	0	4/15	4.0	♀; 10-day embryo
9/14	4.0	♂	4/17	5.7	0
9/16	5.7	0	4/23	4.0	Some development
9/22	4.0	?♀; gonads atypical	4/25	5.7	♂
9/24	5.7	Trace of development	5/1	5.0	♀
10/8	(0)	(2-day embryo)	5/3	6.7	0
10/10	(0)	(♂)	5/9	4.0	♂
10/17	5.0	0	5/11	5.7	0
11/4	14.0	0	5/17	4.0	♂
11/25	(0)	(2-day embr.; incub.?)	5/19	5.7	0
11/27	(0)	(0)	5/25	4.0	♂
12/21	(0)	(0)	5/27	5.7	0
12/23	(0)	(0)	6/11	(0)	(Some devel.; broken)
1/13	(0)	(♂)	6/13	(0)	(Sl. devel.; vasc. area)
1/15	(0)	(?♀; killed very young)	6/19	4.0	0
1/21	4.0	♂	6/21	5.7	0
1/23	5.7	Some development	6/27	4.0	0
2/16	(0)	(Trace of development)	6/29	5.7	0
2/18	(0)	(♀)	7/5	4.0	0
2/25	5.0	♂	7/7	5.7	0
2/27	6.7	0	7/13	4.0	♀
3/5	4.0	♂	7/22	5.0	♂
3/7	5.7	0; abnormal vasc. area	7/24	6.7	0
3/14	5.0	♂	7/30	4.0	0
3/16	6.7	0	8/1	5.7	0
3/22	3.0	4-day embr.; incub.(?)	8/7	4.0	Egg broken
3/24	4.7	Some devel.; incub.(?)	8/9[1]	5.7	0
3/30	4.0	♀			
4/1	5.7	2-day embr.; killed			
Total males and females from stale sperm fertilizations under this (left hand) column of tables 1 to 11 = 56♂♂ : 61 ♀♀.			Total males and females from stale sperm fertilizations under this (right hand) column of tables 1 to 11 = 39♂♂ : 54♀♀.		

*Signifies "not fertile."

[1] Both parents killed for autopsy 9/7; ♂A884 (generic hybrid) tubercular, with worms, and testes partly atrophied; ♀A417, tuberculosis and worms, ovary inactive.

however, of these data on the basis of the extent or degree of staleness. It will be seen that the total for all birds of the three divisions (table 12) shows an excess of females in all three divisions. This is also true for the

TABLE 12

Summary on the relation of stale sperm to fertility and sex

NUMBER OF FEMALE	STALE SPERM FERTILIZATION									NORMAL SPERM FERTILIZATION								
	2 to 4 days*			5 days*			6 and 6+ days*			During experiment			Immediately before experiment[1]			Previous year		
	♂	♀	Inf.	♂	♀	Inf.	♂	♀	Inf.	♂	♀	Inf.	♂	♀	Inf.	♂	♀	Inf.
E361	8	3	0	9	8	1	8	11	5	4	1	0	6	10	0[2]	6	3	3[2]
49	3	9	2	2	5	4	0	4	10	4	9	0	6	7	4	40	44	3
B442	10	11	0	7	5	0	1	1	2	3	0	0	5	11	1			
A684	2	6	0	4	4	0	1	5	5	3	1	0	14	6	0	9	7	0
744	5	1	0	1	0	0				1	0	0	8	2	4			
79	1	5	0	5	5	3	2	2	2	2	1	0	6	13	1	6	7	1
Misc.				0	2	0	0	1	5	3	1	1	19	21	2	8	18	0
Total.....	29	35	2	28	29	8	12	24	29	20	13	1	64	70	12	69	79	7
192[3]	1	8	2	5	7	11	2	0	1	1	1	1	9	5	1	4	12	0
B629[3]	3	2	6	1	1	5	0	0	3	0	4	0	7	3	2	21	24	6
A91[3]	0	2	1	2	3	0	0	2	2				4	8	1	5	7	1
A417[3]	8	4	18[4]	4	1	4	0	0	1	2	2	3	6	8	4	16	16	1
Total[3].....	12	16	27	12	12	20	2	2	7	3	7	4	26	24	8	46	59	8
Grand total.	41	51	29	40	41	28	14	26	36	23	20	5	90	94	20	115	138	15

*All *pairs* of eggs in which the *first* of the pair falls within these limits are included. Also, periods to and including 4.9 are classed with 4-day periods; 5.9 with 5-day periods.

[1] The ten clutches immediately preceding the experiment are included.

[2] The male used in this period was not the same as that used during the experiment.

[3] One or both parents tubercular and this responsible for some of the infertility of the record.

[4] This large group (18) of infertile eggs is associated with the only *generic* hybrid male used in these studies; both parents tubercular at close of the experiment.

group of healthy birds of the three divisions if this group be separated from those birds in which disease developed during the experiment. Moreover, the excess of females is greatest (14♂♂ : 26♀♀) in the division pertaining to the stalest sperm. But does this mean that stale

sperms probably produce an excess of females? This question can be answered only by such control data as are added to this table and by an examination or analysis in which the conditions already known (Whitman (3); Riddle (4), (5), (6), and unpublished data) to affect the sex-ratio in doves and pigeons is considered.

TABLE 13

Showing number of males and females produced at precise periods of staleness and classified as to origin from specific order in pair or clutch

DAYS OF STALENESS	EGG OF CLUTCH			TOTAL
	First	Second	Single	
	♂♂ : ♀♀	♂♂ : ♀♀	♂♂ : ♀♀	♂♂ : ♀♀
0	12 : 6	11 : 12	2	23 : 20
2.2	1			0 : 1
3.3	1			0 : 1
3.9		1		0 : 1
4.0	21 : 21		1 : 3	22 : 24
4.3	1 : 2		1	2 : 2
4.4	1			0 : 1
5.0	21 : 20	1	2 : 2	24 : 23
5.2	1			0 : 1
5.3	2			0 : 2
5.7		15 : 19		15 : 19
6.0	9 : 12	1 : 1	3	10 : 16
6.1		1		0 : 1
6.3	1			1 : 0
6.7		15 : 15		15 : 15
6.9		1		1 : 0
7.0.	2	1		1 : 2
7.7		4 : 9		4 : 9
Total for stale sperm.....	53 : 63	38 : 46	4 : 9	95 :118

Control data for each pair of birds used in the experiment are given under "normal sperm fertilization" in table 12. Three kinds or divisions of control data are available. These concern the sex ratio obtained from *a*, the previous year; *b*, the ten pairs or clutches laid immediately before beginning the experiment with stale sperm; and *c*, the

normal fertilizations which occurred during the experimental period. It will be observed that during the "previous year" (last column, table 12) more females than males were produced by these particular pairs of doves. More females than males were produced by both the healthy group and by the group of birds which became tubercular during the experiment (see footnote, table 12). Also the ten pairs of eggs which were laid "immediately before the experiment" (see next to last column, table 12) show an excess of females for the entire group of birds. The control data obtained from those fertilizations which were effected by normal sperm "during[3] the experiment" show, however, a slight excess of males (23♂♂ : 20♀♀).[4] It thus results that two divisions of the control data, like the three divisions of experimental data, gave an excess of females; one division of control data gave a contrary result. The excess of females (300♂♂ : 350♀♀) in these five divisions deserves a word of comment here. The slight excess of males (23♂♂ : 20♀♀) in the other division which was obtained from normal fertilizations within the experimental period also requires a further statement.

Whitman and Riddle have found that an excess of females is obtained when these doves are made to lay eggs during an extended period of time at an abnormally rapid rate. Tables 1 to 11 show that the stale sperm fertilizations which produced the sexes summarized in table 12 were in most cases fertilizations of eggs laid at an abnormally rapid rate,[5] and that this "crowded reproduction"—or reproductive overwork—was being continued for a period of at least many months. The complete data for the rate of egg laying during the previous year are not given here but the summary given in table 12 indicates that several of the pairs had been strongly "over-worked" during the previous year. As a matter of fact, all of the birds used for this study had previously been made to produce eggs very rapidly during ½ year to 4 years; in some cases, however, many or all of the eggs thus obtained were used for purposes other than incubation, and thus there are no sexes or few sexes recorded for the previous year. The fact of previous "overwork" also applies of course to the period designated "immediately

[3] For some of the pairs used in this study a small amount of data was obtained immediately after the close of the experiment; these data are included. In other words, all of the data of tables 1 to 11 are included.

[4] For the healthy pairs alone the excess is greater (20♂♂ : 13♀♀).

[5] Under normal conditions these doves would have produced probably not more than six to ten pairs of eggs per year.

before experiment." Therefore, the two last-named control periods and the three periods into which the "stale sperm fertilizations" are divided are all characterized as periods during which pairs of eggs were laid in rapid succession. All of these five divisions of the table show an excess of females.

On the other hand, the division which shows an excess of males, called "during the experiment" in table 12, includes practically all of those periods in which the females refused to produce eggs in the absence of the male. To this trial period, sometimes much prolonged, there was then usually added a period during which the male and female remained together before eggs were laid. Only 3 of the 43 offspring of this group (tables 1 to 11) were from eggs laid within 10 days or less after the previous pair of eggs. The remaining 40 were hatched from clutches with a time interval of 11 to 48 days, the average being 22.2 days. It is clear therefore that the division of table 12 which shows an excess of males includes the particular class of eggs which were *not produced at short time intervals*. In contrast with this group, the offspring of known sex which resulted from stale sperm fertilizations (first 3 divisions of table 12) were from eggs laid at quite short time intervals. The 148 clutches which produced such young (tables 1 to 11) have an average time interval of only 6.7 days with a range of variation of 5 to 17 days.

The above analysis of the data sufficiently shows that the sex ratio of the normal sperm fertilizations "during the experiment", and the different ratio found for stale sperm fertilizations, may be adequately accounted for on other grounds than of staleness or normality of sperm. The reason for the association of the *highest percentage* of females with the division of stalest sperm "6 and 6 + days" (table 12) can also be shown to be due to another factor than that of staleness of the sperm. The reason may be briefly stated as follows: The eggs which were fertilized by the stalest sperm were not distributed equally over the various portions of the year. In the early part of the study the parents were left together for longer periods after laying eggs than during the later part of the study; this resulted in a *shorter* interval between the "separation of the parents" (see second and fifth columns of tables 1 to 11) and the next succeeding ovulation in the earlier work. It was later realized that fertility was practically normal with sperm of 4, 5 and 6 days of staleness and that whatever additional data were obtained should preferably be concerned with longer periods of staleness. Such longer periods of staleness were secured by separating the male

from the female very soon after eggs were laid. But these prolonged periods of separation were thus largely concentrated into the *summer months* and after a *longer period of crowded or forced reproduction*. Reference to the right and left halves or divisions of tables 1 to 11 will demonstrate this point. Most of the longest periods of staleness (from which sex data were obtained) found there under the heading "days of separation of parents" will be found in the right-hand sections of the tables. The actual numbers (omitting table 10) are as follows: The left-hand halves of the several tables supply only 4 clutches or pairs of eggs whose sex is recorded in the "6 and 6+ days" column of table 12; while the right-hand halves of the tables supply 22 such clutches.

If one compares the sex ratios of all the stale sperm fertilizations of the right- and left-hand columns separately it will be found that the sum of the (earlier) left-hand columns shows a relatively higher percentage of males, —56♂♂ : 61♀♀; the right-hand columns total 39♂♂ : 54♀♀. As noted above, this later period (the right-hand columns) had been preceded by a longer period of reproductive overwork than had the earlier period and fell, for the most part, in summer.[6] In the work of Whitman and of Riddle to which reference has already been made this general situation was found to raise the proportion of female offspring. The group of offspring listed under "6 and 6+days" in table 12 arose therefore particularly from that portion of the year's record which experience has shown to yield a higher percentage of females, irrespective of staleness of sperm.

In the summaries given in table 12 the two eggs of a pair or clutch are recorded together, i.e., in the same interval column, although the intervals for the two eggs differ by 1.7 days. The results from each clutch have been thus recorded because earlier work has indicated that the females of pure species, and also of some hybrids to a less extent, more often throw males from the first egg of the pair or clutch and females from the second. In any comparison of sex ratios obtained from ring-doves it therefore seems more correct to carry the clutch as a unit. On the other hand, it is desirable to know the number of males and females produced at the actual or specific time intervals represented in the data, and thus make it possible better to judge whether there is a progressive increase of females (or of males) with increased staleness of the sperm. For this purpose table 13 has been constructed and a

[6] A still larger proportion of females than that obtained in summer may be had from the succeeding autumn, and perhaps even of the winter, if the females do not take a period of "reproductive rest."

summary separately presented for the two eggs of the clutch. If above-mentioned factors are considered the data indicate no such progressive increase. For example, it is true that the very longest period of staleness —7.7 days — gave 4♂♂ to 9♀♀; but these embryos are few in number, are from a "selected" group as already noted, and all are from the second egg of the clutch. Possibly the latter point has a bearing on the excess of females present, although table 13 makes it clear that the offspring of the "stale sperm fertilizations" as a group do not show different sex-values for the two eggs of the clutch.[7] The "normal sperm fertilizations," given in the top row of this table, do reflect the situation perhaps more commonly met with in these doves.

In connection with the topic last mentioned above attention should be called to a point in which the data of tables 1 to 11 are imperfect for a complete analysis of sex ratios. This imperfection arises from the fact that because of our wish to exceed the fertilizing period of stale sperm as often as possible, and because of special uncontrollable circumstances, sex has been ascertained in only about one-half (256 sexed, 218 not sexed) of the eggs produced during the period of the experiment. Also, unusually large numbers of the eggs obtained in this study disappeared or were broken, due to the activity of rats, handling, etc.; and in connection with these broken eggs is the circumstance that at least four of the thirteen females produced large numbers of eggs with inadequate shells. This frequently resulted in broken eggs and when the break occurred during incubation and escaped observation for a day or two the egg had to be recorded in tables 1 to 11 as "disappeared." Of still greater importance is the fact that these eggs with inadequate shells produced many embryos which died early—before sex could be ascertained.

We believe, however, that the imperfections of the data noted above still permit a fairly confident conclusion concerning the particular question of the relation of staleness of sperm to sex. The data obtained by us from ring-doves indicate that staleness of sperm had no appreciable or measurable effect on the sex ratios. The sex ratios obtained during the experiment cannot be considered normal, but the observed fluctuations can be accounted for in terms of significant factors previously investigated.

In the several preceding paragraphs the sex ratio obtained from stale sperm fertilizations—95 ♂♂ : 118 ♀♀—has been considered significant,

[7] The sex was obtained of fewer embryos from second eggs than from firsts (84 and 116 respectively), and more second eggs have inadequate shells (see tables.)

and as requiring consideration, not because these numbers are in themselves sufficiently large to establish their significance; but because much other work (Whitman (3) and Riddle, mostly unpublished data) has shown that much larger numbers, as well as considerably smaller ones, with evident regularity yield a similar excess of females under continuous and crowded reproduction.

DISCUSSION

Observations on the vitality of the spermatozoa of different animals demonstrate wide differences among the various classes and species in the length of time the sperm cells retain their fertilizing power. The time during which the spermatozoa retain their power of movement is also widely different for the various animal groups. Among the birds considered alone there are very considerable differences in the time during which the spermatozoa remain active in the female oviduct. The practice of some breeders of turkeys shows that these breeders confidently consider even a single union of the gobbler and turkey-hen sufficient to fertilize all eggs laid by the hen for a period of 3 or 4 weeks. The experiments of Lau (11) show that in the common fowl "some" eggs may be fully fertile for 19 days after removal of the male. After 19 days all eggs were infertile. Payne (12) reported no fertility after 16 days. Barfurth (13) quotes Harvey as stating that "infertility may be calculated to continue twenty to thirty days after separating hens from cocks." The Ontario Agricultural College Report for 1898 records a test in which all eggs were infertile after the 9th day. Concerning the time during which sperm cells of the fowl remain motile there is even wider discrepancy in the observations. Payne reports motile sperm in the oviduct at 56 days, although the number of sperm cells was much reduced after 14 days. Barfurth found no live cells in the fowl's oviduct at a 22- to 24-day period. Lau found active sperm in the sperm ducts of males 24 days after castration.

The results described in the present paper indicate that the spermatozoa of most of the ring-doves studied by us retain their fertilizing power during very close to 8 days. For the pair described in table 11 the time is almost certainly less than 7 days, but the use of the sperm of a generic hybrid male is possibly the explanation of the reduced period in this case. In all of the tests made with the several pairs of doves two eggs only tested fertile at 8 days. Three additional tests at 8 days, one test at 8.7 days, eleven tests at 9 to 13.7 days, and four tests at 14 to 17 days all proved infertile. In all of the above instances the

period of staleness is calculated from the time of separation of the male to the hour at which the egg is laid. It is almost certain that if the egg is fertilized at all this must occur at least 24 hours before laying. All of the figures for staleness of sperm given by us would therefore be made more accurate if one day were deducted from the period as stated.

It is possible that the one notable variation from the usual period of fertility among the pairs of ring-doves studied by us is an individual difference and is not necessarily associated with the hybridity (two genera) of the male parent. Such individual differences probably exist in fowls. Otherwise it is difficult to explain the results obtained on the limits of fertility in the fowl as this has been reported by various authors and cited above.

Only one male of a pure species was successfully tested in the present study. The tests made upon the sperm of this male all showed full fertility but the tests did not exceed a 7.7-day period. Four other attempts to test similar males were unsuccessful. Whether the sperm of males of pure species retains its fertilizing power for a longer period than does the sperm of hybrids is a question which remains undecided. Also, no attempt has been made to learn the length of time during which the spermatozoa of these doves remain motile in the female oviduct. At present we know very little concerning the question whether the sperm of one species of doves will live longer in the oviduct of a bird of the same species than in that of a different species or genus. The results reported in the present paper are, however, practically unaffected by differences possibly involved in that question.

In the introductory paragraphs of this paper reference has already been made to a possible or conceivable relation of stale sperm to abnormal sex ratios. Although staleness or "over-ripeness" of the ova of the frog was one of the observed facts associated with the production of the extremely high percentages of males obtained by Hertwig and by Kuschakewitsch, it has been made fairly clear by King's (14) further study on the toad that the change observed in the sex ratio is possibly associated with a changed moisture-content which occurs during the "over-ripening" of the egg, and not necessarily with any value that may be assigned to "staleness" *per se*. It has been earlier pointed out (6), (15) that the ova of all vertebrates thus far studied in this respect take up moisture from the oviducal or uterine fluids which they meet after extrusion from the ovary. And further, that this change in the state of hydration of the egg affords a possible basis for the interpretation of the abnormal sex ratios thus obtained, since the male and

female-producing ova of doves and pigeons show similar differences of hydration (together with other differences) according to their prospective sex value.

The available evidence therefore gives some reason for attributing a sex-modifying value to staleness of ova which is thus far wholly lacking in the case of the sperm. The positive result[8] of the tests made on the (indirect?) effects of staleness of ova on the sex ratio in the frog (Hertwig, Kuschakewitsch) and the negative tests which we have now obtained on the spermatozoa of pigeons conform to the above-stated expectation. In other words, it was known that staleness of ova favors a change (increase) in the moisture-content of the ovum. Hertwig, Kuschakewitsch and King changed the moisture content of the ova in frogs and toads and apparently obtained abnormal sex ratios; but spermatozoa have not thus far been shown to undergo any definite change in their moisture-content (although this may be wholly due to the great difficulties of making the tests) during their continued lodgment in the female genital tract, and a test of the effect of such delay upon the sex ratio in the case of doves has shown the absence of any measurable effect.

The results of the 213 successful individual tests made by us with stale spermatozoa of ring-doves show no appreciable or measurable effect on the sex-ratio. In conclusion, we would point out that this fact has a further and wider application, since it is now clear that the abnormal sex ratios that have been previously obtained by investigations (Whitman, Riddle) on such doves are not complicated by nor causally associated with staleness of spermatozoa.

SUMMARY

The spermatozoa of the ring-doves used in this investigation retained their fertilizing power during nearly 8 days. This period represents the interval between the hour of isolation of the male and the hour the egg is laid.

Variations in the period of staleness compatible with fertilizing power in various bird species and in individuals of the same species are discussed.

[8] Swingle, (Amer. Nat., liv, 1920) has very recently questioned the validity of the cytological criteria used by previous workers in classifying male and female Anuran larvae. Still more recently, however, Crew (Proc. Roy. Phys. Soc. of Edinburg, xx, 1921) has obtained new evidence of at least a considerable amount of true hermaphroditism in the frog.

No evidence was obtained indicating weakness or modified viability in the embryos obtained from stale sperm fertilizations. Some of the parents used in this study, however, produced numerous eggs within which the embryos were unable to complete their development. The cause of these failures seems to be associated with inadequate egg-envelopes and with deficiences of the ovum which have been made the subjects of further investigation.

Staleness of the spermatozoa did not appreciably affect the sex ratio in the birds studied. Some of the sex ratios obtained during the experiment cannot be considered normal but these abnormal ratios have been shown to be associated with other factors investigated earlier.

The abnormal sex ratios that have been obtained in previously reported investigations on these doves, and any results that may be later obtained from them or from similar birds, are here shown to be probably not measurably complicated by effects due to staleness of the spermatozoa.

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STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

X. Inadequate Egg Shells and the Early Death of Embryos in the Egg

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Some doves and pigeons have been found to produce extraordinarily large numbers of eggs within which the embryos die before hatching. Some of these birds also occasionally produce a fairly continuous series of embryos which die at progressively earlier stages in the later eggs of the series. Doves which yield such series of dead embryos or which produce large numbers of non-viable embryos have been found to enclose some of these in soft shells or in demonstrably thin or inadequate shells and still others of these early-dying embryos in quite normal shells. The occasional egg with soft shell or with inadequate shell therefore becomes a sign of some more deeply seated defect or trouble. The main purpose of this paper is to show that the abnormally high numbers of dead embryos and the inadequate shells are thus loosely yet actually associated.

The present problem is of course a part of the general problem of the causes of death in fetus and embryo. It has a further special bearing upon the enormous annual economic losses which occur from failures of this kind in the poultry industry since it is well known that of the total number of eggs incubated in any country there is a high percentage of eggs either infertile or within which the embryos die before hatching. Probably this is the principal source of avoidable loss in that industry. A part of this infertility and embryonic death is doubtless wholly unrelated to anything in any way associated with inadequate egg shells; but that such egg shells have hitherto undemonstrated associations with these phenomena, and that these latter are utilizable in the poultry industry and elsewhere in reducing the losses resulting from the incubation of unhatchable eggs, will be indicated in the present paper.

In the preceding study of this series it was found by Riddle and Behre (1) that certain ring-dove females produced eggs in which the embryos usually died after a few days of development. Most of these same females were known to be producing some soft-shelled eggs or eggs obviously thin-shelled and easily broken. From this fact it may be inferred that the death of many of the sister-embryos enclosed in intact and apparently good shells was somehow related either to an inadequacy of their own egg-envelopes or to the production by the same parent of occasional thin or inadequate shells.

Since the high proportion of dead embryos noted above occurred in an experiment dealing with the effects of "stale" spermatozoa, and since the dead embryos found there were in greater numbers than have hitherto been observed by us, it seems advisable to supply at once to those not familiar with this material sufficient evidence that there is no necessary or real connection between the staleness of the sperm used and the extraordinarily high death-rate of so many young embryos. Moreover, the sex studies being conducted by the author require the development of all embryos to at least a stage at which their sex may be learned; the cause of failure to reach such a stage is a point of real significance in those studies. In this previous work on sex and fertility there have been encountered phenomena of partial fertility or of restricted life-terms, which it is quite necessary and possible to distinguish from death of embryos due to extrinsic causes or to disease or nutritional disorder in the parent. Much completed work and other studies now in progress have therefore made necessary a search for the real cause of the death of embryos contained in, or in any way associated with, egg shells which are abnormal or inadequate. The statements just made give our own special reasons for a study of this problem.

Presentation of data. The investigation dealing with stale sperm (1) is published simultaneously with the present study and the tabulated data of that paper may be consulted with reference to the production by certain birds of series of eggs within which the embryos died early; also the opportunity is offered there to note the tendency toward shorter life-terms from later or succeeding eggs produced by a particular bird. It can there be observed also that birds which at the end of the record were producing many non-viable embryos had earlier produced large numbers of wholly normal and healthy embryos.

Soon after the conclusion of the above-mentioned study we began an examination of the adequacy of the shells of all of the then-living

TABLE 1

Loss of weight of eggs from different female ring-dove hybrids. In an earlier study (1) few embryos died in eggs of females grouped in top rows; many died in eggs of females grouped in bottom rows; an intermediate number in middle rows

NUMBER OF FEMALE		DATE OF EGG	WEIGHT OF EGG	AVERAGE	LOSS IN MILLIGRAMS PER HOUR AS SHOWN BY SUCCESSIVE WEIGHINGS. NUMBER OF HOURS IN PARENTHESES						Average
					Early		Later		End		
			grams								
Group 1	E361	9/16	9.1				(240)	3.9	(314)	3.9	
		9/23	8.5		(24)	3.3	(26)	3.8	(324)	3.7	
		9/25	9.3		(26)	4.0	(22)	4.8	(310)	4.2	
		10/4	8.0						(336)	3.6	
		10/6	8.3						(293)	6.3	
	A684	9/13	8.4						(335)	4.0	
		9/15	8.8						(308)	4.1	
		9/22	8.7		(19)	3.0	(24)	3.9	(330)	3.5	
		9/24	9.4		(24)	3.9	(26)	4.6	(320)	4.6	
		10/5	8.3						(330)	3.6	
		10/7	8.6	*8.7*					(312)	3.4	*4.1*
Group 2	49	9/16	9.1						(336)	3.7	
		9/18	9.4						(314)	4.5	
		9/25	9.2		(24)	2.8	(24)	3.1	(328)	3.6	
		9/27	7.5		(24)	3.6	(24)	4.2	(308)	4.6	
	79	9/15	7.4						(309)	3.6	
		9/25	8.0		(24)	3.7	(24)	3.1	(327)	4.0	
		9/27	7.1		(24)	3.4	(24)	3.7	(309)	3.8	
		10/8	7.4						(333)	3.4	
		10/10	7.6						(291)	3.5*	
	192	9/12	8.7						(337)	3.7	
		9/14	8.6						(295)	4.7	
		9/20	8.1		(19)	4.0	(24)	3.5	(331)	3.3	
		9/22	9.0		(19)	6.8	(24)	6.3	(312)	5.8	
		10/8	9.2		(148)	4.9			(148)	4.9*	
		10/14	8.0		(48)	2.8	(48)	5.6	(96)	4.2[1]	
		10/16	8.9	*8.3*	(7)	4.8	(48)	5.3	(55)	5.2[1]	*4.2*
Group 3	744	10/5	9.2		(24)	3.3	(25)	2.3[2]	(234)	2.7*	
		10/7	9.2		(24)	4.3	(24)	4.5	(312)	3.9	
		10/14	9.2		(25)	2.8	(46)	3.3	(119)	2.9*	
		10/16	8.9		(25)	7.4	(46)	7.1	(78)	7.2*	

TABLE 1—*Concluded*

NUMBER OF FEMALE		DATE OF EGG	WEIGHT OF EGG	AVERAGE	LOSS IN MILLIGRAMS PER HOUR AS SHOWN BY SUCCESSIVE WEIGHINGS. NUMBER OF HOURS IN PARENTHESES						Average
					Early		Later		End		
			grams								
Group 3	B442	9/22	8.3		(19)	3.0	(26)	1.0[3]	(306)[4]	3.1	
		9/24	8.2		(26)	1.6[3]	(24)	5.0	(192)[4]	5.2*	
		10/1	8.2		(23)	2.7	(23)	2.7	(211)	2.8*	
		10/3	8.1		(23)	5.2	(22)	4.5	(319)	4.8	
		10/10	8.2		(8)	2.9	(24)	3.4	(219)	3.1*	
		10/12	7.3		(24)	5.1	(20)	5.0	(320)	5.0	
	B629	9/13	8.6						(273)	5.1	
		9/24	8.6		(26)	6.5	(22)	5.5	(304)	5.8	
		10/5	9.0		(25)	3.6	(24)	4.8	(230)	5.3*	
		10/7	9.5		(24)	11.5	(25)	10.8	(312)	10.1*	
	A60	9/24	8.8		(22)	4.6	(27)	5.0	(311)	4.6	
		9/26	9.3		(22)	9.3	(27)	9.4	(310)	8.1	
		11/26	9.5		(189)	4.5	(68)	6.2	(306)	4.3	
		11/28	10.2	*8.8*	(147)	9.7			(147)	9.7[1]	*5.2*

* Produced embryos which died before hatching. All other eggs hatched.

[1] Embryos killed at early age in an experiment (where they were placed by mistake); the possibility of their living to end of incubation not tested.

[2] Shell reinforced with tape which lessened rate of loss.

[3] Egg left at room temperature (not incubated) during this period.

[4] Exclusive of time kept at room temperature.

birds used in the earlier study. Much earlier experience with soft-shelled and thin-shelled eggs had shown that those eggs with least shell material lost weight most rapidly. For example, an egg that had a very thin deposit over one-half its surface, and shell membrane only over the other half, showed a rate of loss at room temperature of 0.121 to 0.145 gram per hour for 17 hours. This was 84 times the rate at which an egg with presumably normal shell lost weight under the same conditions. Practically all intermediate rates of loss have been found and measured and it seems that, without special consideration of such data, we may here treat relative rates of loss from eggs as a measure of their relative permeability to water or water vapor. During the first day after the egg is laid the loss is in fact essentially nothing else than a loss of water; though later there is of course a perceptible loss through CO_2-production and a gain through

O_2-fixation; but these latter tend to balance each other, and the unsatisfied balance is essentially equalized between different eggs by the circumstance that embryos are present in all of the eggs compared. Eggs containing embryos dying prematurely were weighed and opened very soon after the death of the embryo so that rates of loss as measured indicate the rate while a living embryo was present. This rate is reduced after the death of the enclosed embryo. For most of the embryos the rate of loss was obtained at five successive periods; the rates obtained for three of these periods are found in table 1.

An examination of tables 1 to 11 of the earlier paper (1) will show that those females which we have here grouped at the top of table 1 had produced no eggs with soft shells; none of their eggs were later broken, and the eggs laid by them showed the smallest proportion of dead embryos. Similarly those females placed in group 2 of table 1 will be found to have shown a slightly greater proportion of these abnormalities. Group 3 of the table includes those females which in the earlier investigation produced relatively the greatest number of soft-shelled eggs, broken eggs and dead embryos.

A comparison of the average egg weights and average rates of loss of water by the three above-mentioned groups makes it clear that the group which produced most dead embryos—in both the earlier and the present study—also has the highest rate of loss[1] of water (5. 2 mgm. per hour); and the group with lowest rate of loss (4.1 mgm. per hour; egg weight = 8.67 grams) corresponds to the group that produced—in both the earlier and the present study—fewest dead embryos.

If one looks for evidence of a high rate of loss associated with the death of specific or individual embryos within group 3 of table 1 the data are confusing and quite inconclusive. Other factors than rate of loss are plainly involved. All individual eggs with high rate of loss do not die and all individual eggs with lower rates of loss do not survive and hatch. It is clear only that the *group* of birds which experience had

[1] The actual rate of weight-loss from the egg is of course much influenced by the temperature to which it is exposed as well as by the thickness of shell. In order to show this the first two eggs of ♀ B442, shown in table 1, were left during 26 hours at room temperature (about 20°C.) with the result that the loss was reduced to about one-third normal. All doves incubate inconstantly until the second egg of the pair is laid. Of course the rate of loss is related to the amount of shell surface; the latter, however, can be measured only with difficulty and since surface bears a close relation to weight, the weight of each individual egg is given in the tables.

shown to produce occasional soft-shelled eggs and many dead embryos in apparently good shells is the group of birds whose eggs have the thinnest shells. Also that at least some of the birds which produce thin shells occasionally yield shells which are thicker than is normal; this fact emphasizes the disordered condition of these oviducts.

A few months later a second lot of data was collected. At that time there were six birds in our collection which had recently produced one or more soft-shelled eggs. These six birds were taken for careful study (table 2) and three females producing only eggs with presumably normal shells were taken as control (table 3). All eggs produced by these nine birds during a period of 5 weeks were supplied with the most careful incubation under the most trustworthy birds; their rates of loss were twice determined and the age attained by their enclosed embryos recorded. The data of table 2 contrast with those of the control (table 3) in quite the same way that group 3 of table 1 has been seen to contrast with group 1 of that table. All of the six birds of table 2 gave one or more dead embryos, and the lowest average rate of loss from a bird of this group was higher than the highest average for the control.

During the year that has elapsed since the above data were obtained many data have been obtained from time to time. All of the latter data have very fully confirmed those given in tables 1 to 3. The normal rate of loss for eggs of these ring-doves lies between 3.0 and 4.0 mgm. per hour. Birds which produce soft-shelled or obviously thin-shelled eggs produce also many others with apparently normal shells whose rate of loss exceeds 6.0 mgm. per hour; and an unmistakably high proportion of the embryos die before hatching. Here also the dead embryos are found from eggs of low as well as of high rate of loss. The tabulation of these records would require considerable space and would add nothing except volume to the better-controlled data already presented.

One further fact concerning the origin of inadequate egg shells is made clear by the data, namely, that the inadequate shells are much more often found on the second egg of a pair than on the first. The eggs are laid 2 days (40 hours) apart and to make the above comparison possible the date of laying is given in tables 1 to 3. A summary of this situation is given in table 4, where it can be seen that 47 first eggs of the pair or clutch had an average loss of 3.7 mgm.; 46 second eggs an average loss of 5.7 mgm. A similar difference is found in all

TABLE 2

Rate of loss from eggs and survival of embryos from all eggs laid during a period of five weeks by all ring-dove females known to be producing some eggs with thin shells. All eggs incubated under birds

NUMBER OF FEMALE	DATA ON EGGS		RATE OF LOSS (IN MILLIGRAMS) PER HOUR (5-DAY PERIODS)			STAGE ATTAINED BY EMBRYO
	Date laid	Weight	First period	Second period	Average	
B629	4/5	8.4	8.5*	8.0	8.3	Hatched
	4/22	7.5	5.4	4.6	5.0	Hatched
	4/24	8.5	9.1	7.8	8.5	Dead, 11-day embryo
	5/1	8.2	5.6	4.6	5.1	Hatched
	5/3	8.5	11.6*	9.3	10.5*	(Dead?)[1] 11-day embryo[2]
	Av.	*8.2*	*8.0*	*6.9*	*7.5*	
B442	4/6	7.9	5.0		5.0	Dead, 3.5-day embryo
	4/15	8.3	2.6	2.5	2.6	Dead, 7-day embryo
	4/17	8.5	5.7	5.7	5.7	Hatched
	4/23	7.7	3.0		3.0	Dead, 4.5-day embryo
	4/25	8.4	6.6		6.6	Dead, 3-day embryo
	5/2	8.3	2.8		2.8	Dead, 1-day embryo
	5/4	8.5	6.4		6.4	Dead, 1-day embryo
	Av.	*8.2*	*4.6*	*4.1*	*4.6*	
K465	3/31	8.5	4.6	4.4	4.5	Hatched
	4/2	8.7	6.6	5.6	6.1	Hatched
	4/12	8.5	3.5	3.2	3.4	Hatched
	4/14	8.6	5.2	4.6	4.7	Hatched
	4/21	7.8	3.8	2.6	3.2	Dead, 13-day embryo
	4/23	Soft shell			(?)	
	4/30	6.9	3.8	3.6	3.7	Hatched
	5/1	8.2	8.1		8.1	Dead, 2.5-day embryo
	Av.	*8.2*	*5.1*	*4.0*	*4.8*	
A60	4/15	8.1	3.5	4.3	3.9	Dead, 14-day embryo
	4/17	9.3	5.4	5.6	5.5	Hatched
	4/24	8.7	3.9	4.5	4.2	Hatched
	4/26	10.1	6.9	7.5	7.2	Hatched
	5/4	8.9	4.0	4.5	4.3	Hatched
	5/6	10.2	6.3	6.4	6.4	Hatched
	Av.	*9.2*	*5.0*	*5.5*	*5.3*	
P431	4/4	8.7	3.8	4.2*	4.0	Hatched
	4/6	9.1	9.6*		9.6*	Live (?), 6.5-day embryo[2]
	5/1	8.5	3.9	3.4	3.7	Dead, 13.5-day embryo
	5/3	9.4	7.1	6.2	6.7	Hatched
	Av.	*8.9*	*6.1*	*4.6*	*6.0*	
K954	4/11	9.1	8.9	8.8	8.9	Dead, 5.5-day embryo
	5/4	9.2	4.5		4.5	Dead, 2.5-day embryo
	5/6	10.0	(27.5)*		(?)	Live, 4-day embryo[2]
	Av.	*9.4*	*6.7*	*8.8*	*6.7*	
	Average for group = 5.6 mgm. per hour					

* Egg slightly broken; repaired with tape.

[1] It is possible that a further slight break in shell on 5/14 was cause of death.

[2] Egg broken.

of the five groups which are included in this average.[2] It is also true that eggs from birds which are laying presumably only normal shells show

TABLE 3

Rate of loss from eggs and survival of embryos from all eggs laid during a period of 5 weeks by females thought to be producing eggs with normal shells (control data for table 2)

NUMBER OF FEMALE	DATA ON EGGS		RATE OF LOSS (IN MILLIGRAMS) PER HOUR (4–7 DAY PERIODS)			STAGE ATTAINED BY EMBRYO
	Date laid	Weight	First period	Second period	Average	
P583....	3/31	9.0	4.0	4.2	4.1	Hatched
	4/2	9.4	5.2	5.5	5.4	Hatched
	4/10	8.0		3.3	3.3	Hatched
	4/12	9.0		4.6	4.6	Hatched
	4/18	8.8	3.5	4.0	3.7	Hatched
	4/20	9.4	4.5	4.2	4.3	Hatched
	4/26	8.6	3.2	3.5	3.3	Hatched
	4/28	9.5	4.5	5.1	4.7	Hatched
	Av.	*9.0*	*4.2*	*4.3*	*4.2*	
P728....	3/31	8.6	2.9	3.2	3.0	Killed or died at 12 days
	4/2	9.2	3.2	4.0	3.6	Killed or died at 10 days
	4/10	8.3		3.1	3.1	Hatched
	4/12	9.0		3.4	3.4	Hatched
	4/18	9.2	5.0	5.2	5.1	Hatched
	4/20	9.6	4.8	5.0	4.9	Hatched
	4/26	9.2	3.3	3.8	3.5	Hatched
	4/28	10.1	3.6	5.5	4.5	Hatched
	Av.	*9.2*	*3.8*	*4.2*	*3.9*	
P720....	3/29	6.8	2.8	2.9	2.8	Hatched
	3/31	7.1	3.9	3.8	3.9	Hatched
	4/11	7.3	2.5	2.7	2.6	Hatched
	4/13	7.4	4.0	4.0	4.0	Hatched
	4/20	6.6	3.0	3.1	3.0	Hatched
	4/22	7.6	4.2	4.3	4.2	Hatched
	4/29	6.5	3.8		3.8	Dead 5-day embryo
	5/1	7.3	6.3		6.3	Hatched
	Av.	*7.1*	*3.8*	*3.5*	*3.8*	
	Average for group = 4.0 mgm. per hour					

[2] The second egg of the pair is usually, but not always, larger than the first. With a slightly greater surface area it should of course show a proportionately higher rate of loss. An inspection of the data obtained from birds which produce thin-shelled eggs clearly shows, however, that the second eggs have disproportionately high rates of loss.

much less difference between the shells of the two eggs of the pair than do the eggs of birds which are known to be producing some thin-shelled or soft-shelled eggs. Thus the first eggs of twelve pairs in table 3 show a rate of loss of 3.4 mgm. per hour and the seconds 4.6 per hour; and sixteen pairs from table 2 have an average loss of 3.9 mgm. per hour for firsts while the seconds show a loss of 7. 1 mgm. per hour. The data of the tables make it clear, however, that some first eggs, and also some single or unpaired eggs, are provided with inadequate shells, and that embryos die in all classes of eggs.

As measured by relative rates of loss the second egg of the pair has been shown to have the thinner shell. The data collected in table 5 show that the second eggs also contain less than their proper proportion of dry shell material. The table includes all of the control[3] data

TABLE 4

Showing that the second egg of the pair or clutch usually has a less adequate shell (as measured by rate of loss of weight by egg) than the first of the pair

SOURCE OF DATA	FIRST OF PAIR			SECOND OF PAIR		
	Number of eggs	Weight of eggs	Rate of loss	Number of eggs	Weight of eggs	Rate of loss
Group 1, table 1	5	8.4	3.7	5	8.9	4.5
Group 2, table 1	7	8.4	3.7	7	8.3	4.6
Group 3, table 1	8	8.8	3.6	8	8.8	6.8
Table 2	15	8.3	3.9	14	8.4	7.1
Table 3	12	8.1	3.4	12	8.7	4.5
Average (weighted)		8.4	3.7		8.6	5.7

obtained in the two next following investigations of this series of papers (2), (3), and are given here because the condensed tabulations of those papers prevent their inclusion there. In only one pair of eggs shown in table 5 was the second egg smaller than the first and in three pairs the two eggs were of equal weight; but the data show thirteen pairs in which there was absolutely less shell material on second eggs than on the corresponding firsts. Fewer figures are at hand for the weight of ash and these are less indicative of a difference than are the figures for dry shell material. The percentage of ash in the dry shell material seems to favor neither first nor second egg of the pair. The more com-

[3] Three birds either ceased producing eggs or began producing abnormal eggs (see Riddle and King (3) table 3) soon after dosage with atropine. The "control" eggs obtained in this latter period are here excluded.

TABLE 5

Showing amounts and relative amounts of shell material found on the first and second eggs of ten normal ring-doves. (The eggs are all in pairs; the first of the clutch is written first)

WEIGHT IN GRAMS			PERCENTAGE OF		WEIGHT IN GRAMS		BASES (AS $CaCO_3$) IN ASH	PERCENTAGE OF SHELL IN EGG WEIGHT
Egg	Shell (dry)	Ash	Shell in egg weight	Ash in Shell	Egg	Shell (dry)		
8.2	0.4388	0.2348	5.349	0.5351	9.5	0.4473	94.95*	4.719
9.2	0.4655	0.2422	5.061	0.5202	9.3	0.4255	95.43	4.583
8.4	0.4374	0.2346	5.211	0.5364	9.6	0.4528	95.67	4.711
8.9	0.4405	0.2343	4.926	0.5319	10.4	0.4969	95.55	4.761
7.0	0.3311	94.69*	4.734		9.3	0.4388	95.66	4.730
7.1	0.3755	95.76	5.268		10.4	0.4788	95.77	4.584
9.4	0.4758		5.070		8.2	0.4031	95.17	4.888
10.8	0.5067		4.705		8.8	0.4324	96.22	4.909
9.5	0.4599	0.2470	4.816	0.5372	9.2	0.4619	95.55	5.046
11.3	0.4655	0.2513	4.132	0.5398	9.5	0.4631	95.83	4.892
9.2	0.4287	95.83*	4.653					
10.3	0.4429	95.60	4.316		8.4	0.4689		5.584
					9.3	0.4497		4.848
9.6	0.4523	95.77	4.711		8.3	0.4558		5.517
10.6	0.4686	95.92	4.403		8.8	0.4166*		4.750
8.8	0.4356	95.17	4.932		8.6	0.4361	95.51	5.082
10.6	0.4362	95.49	4.117		8.8	0.3918	95.13	4.472
9.7	0.4635	93.86	4.759					
10.5	0.3891	95.32	3.723		8.8	0.4285	95.81	4.885
					9.4	0.4813	95.40	5.115
7.0	0.3440	0.1856	4.936	0.5395	8.6	0.4644	95.81	5.427
8.1	0.3619	0.1925	4.452	0.5319	9.2	0.4170	95.59	4.532
					8.4	0.4513	95.30	5.354
7.9	0.3704[1]	0.1891	4.705	0.5105	8.9	0.4206	96.20	4.740
8.9	0.3930[1]	0.2054	4.412	0.5226				
8.0	0.3733	0.1910	4.694	0.5117	8.9	0.4183		4.700
8.9	0.3854[1]	0.2045	4.336	0.5305	9.0	0.4370		4.858
					8.6	0.4114	95.75	4.798
7.9	0.3995	0.2157	5.049	0.5399	8.6	0.4243	95.88	4.945
8.6	0.4245	0.2271	4.928	0.5350				
8.0	0.4034	0.2160	5.035	0.5354	8.8	0.4302		4.883
8.8	0.4252		4.853		9.3	0.4165		4.486
7.5	0.3420	95.54*	4.559		8.4	0.3959		4.725
8.3	0.3860	95.71	4.660		9.0	0.3981[1]		4.413
					8.7	0.4148	96.28	4.785
8.0	0.3752	95.83	4.695*		8.9	0.4130	95.74	4.634
8.7	0.4010	95.93	4.627					
7.8	0.3445	94.30	4.404					
8.3	0.3700	95.33	4.451		8.1	0.3904	96.12	4.832
					9.1	0.4141	95.77	4.543
7.0	0.3171	95.84	4.518		8.9	0.3879	95.64	4.363
8.2	0.3604	96.09	4.603		9.3	0.3780	95.47	4.057
7.7	0.3515	96.20	4.558		8.9	0.3692	95.70	4.126
8.3	0.3566	96.29	4.310		8.9	0.3583	95.33	4.031
8.8	0.4510	0.2299	5.135	0.5097	8.3	0.4542		5.489
9.5	0.4778[1]	0.2538	5.021	0.5313	8.3	0.4506		5.421
8.6	0.4477	0.2412	5.183	0.5388	7.7	0.4277	96.11	5.565
9.3	0.4799	0.2603	5.148	0.5425	8.1	0.4562	95.68	5.639

* Percentage of bases calculated as $CaCO_3$ in shell.

[1] Traces of shell membrane present.

plete comparison of the percentages of alkaline bases (calculated as as $CaCO_3$) for the two eggs of the clutch indicates that the ash of the second egg contains quite as much of these bases as is found in the ash of firsts; the relative amount of shell materials from which these figures were derived seems also nearly normal. Twenty-five first eggs with an average weight of 8.5 grams had 0.4091 gram of shell, of which 95.52 per cent was $CaCO_3$; while the same number of second eggs had an average weight of 9.1 grams with 0.4175 gram of shells, of which 95.70 per cent was $CaCO_3$.

The numerous figures for the percentage of total weight which is represented by shell requires detailed examination, as well as a summary such as is given below, in order to discover a tendency on the part of second eggs of some of the birds to show significantly[4] lower proportions of shell material. The 42 first eggs of the table have an average weight of 8.5 grams; their dry shells a weight of 0.4155 gram; and their shells average 4.903 per cent of the total weight. The 42 second eggs have an average weight of 9.2 grams, their dry shells average 0.4246 gram, and their shells average 4.658 per cent of the total egg weight. This definitely lower percentage of the total egg weight represented by the shells of the second eggs is probably significant. The several instances noted above in which the smaller first egg of the pair received absolutely more shell than its associated second are certainly significant. Altogether these results are of additional value because all of the eggs concerned were derived from birds which produced only eggs with presumably normal shells—they certainly produced no obviously thin-shelled eggs.

DISCUSSION

Our search for the cause of the obscure defects and disturbances here described naturally led us to inquire whether the defect is a matter of inheritance. Among the ring-doves, which have been the principal material studied by us, we do find a relatively large number of affected birds among the descendants of a particular ancestral pair of doves; but the disturbance has also been found in offspring of various and widely different ancestry. The trouble may arise, moreover, in the later eggs of an individual bird which has produced many and

[4] Since the second eggs are slightly larger their shells may be of equal thickness with those of first eggs and yet show a *slightly* lower proportion of shell weight to egg weight.

only apparently normal shells and practically only viable embryos during one, two or more earlier years of her life. This striking fact is opposed to the assumption that lethal factors in particular or heredity in general are largely, if at all, involved. Nearly all of the available data indicate that the disorder has a temporary and physiological basis.

The disturbance has been observed in many kinds of hybrids and in pure species of several different genera and families of pigeons. Among these latter are Columba (2 species), Turtur, Spilopelia, Stigmatopelia and Zenaidura. It is well known that soft and inadequate shells occur in the common fowl and although we have not made a special study of the early-dying embryos of that bird it may be safely assumed that conditions there are in the main similar to those among pigeons. Among poultrymen it is rather generally assumed that an egg with a soft or thin shell merely signifies that the egg was expelled prematurely from the oviduct. That this premature expulsion of the egg does sometimes occur is unquestionable for both fowls and pigeons. In pigeons, however, it is certainly exceedingly rare. Furthermore, in pigeons, the definite hour or time of egg-laying has permitted us to know positively in these cases that the egg was in the oviduct during exactly the normal number of hours.

The special deficiency of the shells of second eggs of the pair as described above would seem to indicate that the secretion of an adequate shell is in some way facilitated by the inactivity of the shell gland during 6 or more days prior to the laying of the first egg. The secretion of the second shell follows soon after the conclusion of the shell secretion for the first—within 10 to 15 hours—and in each case the gland is active during about 30 hours. In other words, the data suggest that the bird's store of shell-forming materials is depleted or diminished before the secretion of the second shell is complete. Since nearly all of the dry shell is calcium carbonate it seemed reasonable to suppose that if such deficiency in shell-forming materials really exists it implies a deficiency of calcium which might in turn be overcome by extra feeding of soluble calcium salts. The latter possibility has been tested with a negative result in the next following paper of this series (2).

Wheeler (4) and Buckner and Martin (5) obtained some thin-shelled eggs from fowls after prolonged reduction of calcium intake, and showed that calcium is then removed from the bones for shell formation. The latter authors obtained no shell-less eggs and conclude

that "the lack of calcium is not the fundamental cause of their formation." The data obtained by Riddle and Hanke (2) seem to make this conclusion fairly certain for pigeons.

It is quite possible, of course, that not a deficiency of calcium but an unbalanced proportion of this element in relation to other elements, —phosphorus, potassium, sodium, etc., is the basis of the disorder. It is clearly possible also that the disorder is wholly unconnected with any of these or with any other substances necessary to normal nutrition. The possibility of a vitamine or other nutritional deficiency is, however, further suggested by the observation that most of the aberrant shells and embyros are produced by females which have twisted and abnormal keel bones—a condition readily suggestive of rickets. A later publication by Riddle and Rose will show that whatever the nature of this disturbance it is not corrected by the administration of any or all of the vitamines as these are prepared from yeast, skim-milk powder or spinach; nor as they exist in their normal state in orange juice, tomato juice and cod liver oil. A thorough investigation of the possible relation of nutritional deficiency to the production of thin shells and early-dying embryos is now in progress.

SUMMARY

Individual female pigeons which occasionally produce soft-shelled eggs and obviously thin-shelled eggs may produce other eggs with quite normal or even with unusually thick shells. A very high proportion of the embryos which arise in all these groups of eggs die before hatching.

The production of inadequate shells and the early death of the embryos are thus causally associated, although the relative inadequacy of a particular shell is but loosely correlated with the death of the particular embryo contained within it. An unknown and more deeply seated cause is responsible for both the occasional inadequate or irregular shells and the numerous early deaths of embryos.

Experience indicates that among pigeons the thin shells and associated early-dying embryos often occur after a long series of normal shells and viable young have been produced. This too when the same male is used throughout; the sperm cells are probably not in any way responsible for the result. When female pigeons are made to produce eggs at an abnormally rapid rate it sometimes occurs that series of embryos show the attainment of more advanced age by the earlier

embryos of the series and progressively earlier embryonic death to the end of the series.

The relative inadequacy of the shells can be measured by the relative rates of loss of water vapor through the shells. By this means of measurement the second eggs of the pair or clutch usually have the thinner shells. This is particularly true for the eggs of birds which produce some eggs with inadequate shells.

The measurement of actual amounts of dry shell material, of ash and of total inorganic bases in the ash of many eggs with presumably normal shells lends support to the view that the second eggs of the clutch are more likely to receive a slightly reduced relative amount of shell material.

Some considerations would suggest that the organism's available supply of calcium is depleted before the bird has completed the formation of two shells in rapid succession. The early death of the embyros seems, however, to indicate that something in the ovum (germ) is in disorder almost or quite simultaneously with the disordered functioning of the oviduct. Several possible nutritional deficiencies have been investigated. The real cause of the disorder in ovum and oviduct is quite unknown.

Whatever the nature of the cause of the inadequate shells and the early death of associated embryos it is clear that among pigeons, and probably also in several or all branches of the poultry industry, many individual birds which persistently fail to produce viable young may be identified and their eggs eliminated from incubation tests through the observation that they produce some eggs with soft or inadequate shells.

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STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

XI. Effects of Feeding Soluble Calcium Salts upon Reproductive Secretions and upon the Total Inorganic Constituents of the Egg Shell

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In an earlier study (1) one of us has shown that apparently normal, though really inadequate, egg shells are responsible for the early death of an important fraction of bird embryos. Any considerable experience in working with such eggs readily leads to the conclusion that the shells are too thin and probably do not contain the normal amount of inorganic shell-substance. Since it is known that calcium carbonate forms rather more than 90 per cent of the moist shell there exists the possibility that a lack of soluble calcium in the food is associated with the production of these thin and deficient shells. The present study is chiefly an attempt to learn whether the addition of calcium lactate and calcium lactophosphate to the usual diet of ring-doves results in any increased deposition of inorganic matter in the shell.

Practically all investigations on the subject have shown an increased calcium excretion in the urine of mammals following the ingestion of inorganic lime salts. Givens (2) found the same in using calcium lactate. Earlier studies on the calcium balance under calcium lactate feeding usually demonstrated calcium retention following fairly heavy dosage. Positive results were reported for the human subject by Berg (3) who administered 3 grams daily and by Voorheve (4) who used 15 grams daily. Mendel and Givens (5) obtained a slight positive calcium balance in dogs when dosage was raised to 3.53 grams daily. Determinations of the amount of calcium in the blood following calcium lactate feeding suggest that among mammals an increase of blood calcium can be thus obtained in some species, but not in others; or possibly in the latter species only in individuals with abnormally low blood calcium. Boggs (6) obtained a decided increase in whole

blood of the dog. Halverson, Mohler and Bergeim (7) found that the calcium values of human serum are usually little affected but were increased to normal in cases of uremia and nephritis; also that the calcium excretion in the urine may be increased in certain diseases but not in others. Denis and Minot (8) failed in most cases to find an increase in the plasma of men, cats and rabbits. They further state "that in cats and rabbits where the initial concentration is low it is sometimes possible to greatly increase the amount of calcium in the plasma."

Effects of calcium lactate feeding upon reproductive functions have been reported by Emmerich and Loew (9) who stated that female mice, guinea pigs and rabbits respond to dosage by increased numbers of pregnancies and higher average numbers of embryos. Pearl (10) has made a partial report upon the result of feeding calcium lactate and also calcium lactophosphate to growing fowls. It is stated that the rate of growth of young female chicks was much increased, and that their egg laying records (later?) were greatly increased. In the latter study the dosage used seems to have been considerably larger than we have employed. In our own study there was no increase in the rate or number of ovulations.

Since the present study was concluded Buckner and Martin (11) have published results which are of very considerable interest in connection with the chief topic of the present paper. During several months Buckner and Martin withheld from laying hens all of the inorganic calcium supply which fowls usually obtain from limestone, oyster-shell grit, etc. They conclude: That such hens will continue to lay eggs until there is a general depletion of Mg, P and Ca in the bones; that egg production was much decreased; that the percentage composition of the egg shells thus obtained was not materially altered but a general thinning of the egg shells occurred; and that "since no shell-less eggs were laid it would indicate that the lack of calcium is not the fundamental cause of their formation." These investigators freed and ashed the egg shells of the fowl, much as we have done in the dove, and in addition made determinations of Ca, Mg and P in the ash. They found the calcium of the ash, calculated as CaO_2, to be about 98.0 per cent of the total ash; MgO, about 0.71 per cent; P_2O_5, about 0.70 per cent; with other undetermined elements therefore very small in amount.

From a study of calcium and phosphorus metabolism in cattle, Meigs, Blatherwick and Cary (12) obtained evidence that nervous disturbance, connected with the collection of urine, feces, etc., has a distinct effect

upon the cow's assimilation of calcium. Our own study involved this possible disturbance of shell formation but earlier work had shown that yolk and albumen secretion were certainly not thus modified.

Materials. It would at first seem an easy matter to obtain a definite answer as to whether feeding calcium salt results in the production of increased amounts of shell-substance. But in ring-dove eggs the actual variations of egg size and surface—upon which the amount of shell substance normally depends—and the possibility that the calcium salts used may themselves introduce further variations in some of the secretions—upon which again total egg size depends—unite in making the problem much less simple. We have thought it necessary to study series of eggs obtained from ten different female ring-doves, and to use three different quantities or degrees of dosage. It thus results that some at least of the several necessary measurements have had to be made upon 140 eggs. Control data, before and after calcium feeding, were obtained from 88 eggs; 2 eggs were obtained from the lightest dosage (0.113 gram, once daily, ♀48); 28 eggs were obtained from the same amount given twice daily and 20 eggs from heavy dosage (0.226 gram twice daily). Of the dosage mentioned 64 per cent was calcium lactate and 36 per cent was calcium lactophosphate.

The ring-doves used in these experiments had an average weight of 170 grams. The total daily amount of calcium, calculated as calcium lactate, in the lightest dosage was therefore about 0.45 gram per kilo body weight. Our lightest dosage was higher than that used in any mammalian studies with which we are familiar; but since these small birds were losing more than $\frac{1}{4}$ gram of Ca in the shells alone of the two eggs produced each 8 or 10 days, the dosages selected were considered advisable. The birds were caught twice daily and capsules containing the calcium salts were placed, usually with very slight resistance, well into the throat with artery forceps. There was no regurgitation of dosage.

Full and complete measurements were not made on all of the 140 eggs. The separation of the shell, piece by piece, from the shell membrane of the thoroughly steamed egg was a most tedious and time-consuming task; this separation was omitted for all of the later control series (after dosage). The pressure of other work also prevented the ashing of a number of prepared shells. Nevertheless, we believe an adequate amount of data is at hand. Since eleven or twelve tables would be necessary to give the details and necessary summaries of these data we have thought it advisable to present only the averages and summaries here grouped within four tables.

Presentation of data. The first point made clear by the tabulated data is that during the calcium feeding there was, in most cases, a progressive decrease in total egg size. For some individuals this continued even after dosage was discontinued. Since the absolute amount of shell-substance is closely correlated with the amount of egg surface, and this latter with egg size, it is clear that the measurements of shell weights and total ash must be interpreted in the light of this progressive decrease of egg surface. We have not attempted a direct measurement of egg surface; but, as an indirect measurement of the amount and direction of changes in surface, we have made complete records of egg weights. Although two of the ten treated females show an increase instead of a decrease in egg size, the fact remains that the average egg size was reduced; and unless the amount of inorganic material deposited per unit of surface was increased by the calcium dosage, a smaller amount of dry shell material and inorganic substance may be expected from shells obtained during the dosage period. The data from individual females show that of the eight whose egg size decreased under dosage, six show a decrease and two an increase in shell weight under dosage Of the two birds showing increase of egg weight under dosage one shows increase in shell weight while the other shows perhaps a slight decrease (tables 1 to 3). All of the averages of table 4 indicate a reduction of egg size under dosage, and also a decrease in absolute amounts of dry shell-substance. Such a result concerning the dry shell-substance found for control and treated periods indicates that the calcium feeding had little or no influence upon the absolute amount of substance utilized and laid down by the shell glands of the birds.

An important consequence of this diminution of egg size under dosage is an expected rise in the proportion of shell weight to total egg weight, since the smaller sphere or ovoid has a higher proportion of surface in relation to mass than a larger sphere. That this is of significance for even the relatively slight differences found here is made quite clear by the details of our data for 17 pairs (or clutches) of eggs used for "control" (before dosage). In 16 of these 17 pairs the shell weight of the smaller egg was the higher percentage of the total egg weight. But if comparison is made between the eggs (averaging smaller) of the "dosage period" and those of the earlier "control period" it will be found that the (smaller) treated eggs tend to have rather lower than higher values for shell material in proportion to total egg weight (next to last columns, tables 1 to 4). This necessary consideration of the results also gives therefore no indication that the calcium feeding was able to

increase the proportion of shell-substance. The proportion of dry shell material is nearly equal to, or slightly less than, the usual or expected amount.

The results for total ash are essentially comparable with those just stated for dry shell weights. The absolute amounts of ash were usually decreased (tables 1, 3, 4). Taking proper account of differences of

TABLE 1

Effects upon various egg-structures obtained by feeding calcium lactate and calcium lactophosphate to female ring-doves (no. A622, upper division; 907, middle division; A347, lower division of table)

TREATMENT	DATE OF EGGS	NUMBER OF EGGS	AVERAGE WEIGHT IN GRAMS					PERCENTAGE RELATIONS OF PARTS			
			Egg	Yolk	Albumen	Shell (dry)	Ash	Yolk to egg	Shell to yolk	Shell to egg	Ash to shell
Control....	10/28–11/7	4	8.685	2.199	6.040	0.4456	0.2365	25.34	20.31	5.137	53.09
Smaller dosage...	11/13–11/24	4	8.243	2.155	5.682	0.4050	0.2191	26.16	18.84	4.918	54.08
Heavy dosage...	12/ 1–12/20	5	7.605	2.113	5.112	0.3801	0.2046	27.78	18.04	5.005	53.83
Control....	12/26–1 /14	5	7.620	2.125				27.84			
Control....	10/28–11/ 9	4	9.064	1.932	6.692	0.4641	0.2463	21.31	24.06	5.122	53.06
Smaller dosage...	11/18–11/20	4	9.118	2.041	6.608	0.4698	0.2536	22.34	23.11	5.153	53.66
Heavy dosage...	12/ 8–12/20	4	9.110	1.959	6.671	0.4797	0.2583	21.52	24.54	5.266	53.84
Control....	12/27– 1/8	4	9.206	1.972				21.40			
Control....	11/ 2–11/13	4	8.406	1.961	6.064	0.3805	0.1975	23.26	19.63	4.536	51.88
Smaller dosage...	11/21–12/3	4	8.019	1.884	5.775	0.3604	0.1913	23.46	19.19	4.493	53.05
Heavy dosage...	12/12–12/14	2	8.084	2.028	5.698	0.3578	0.1897	25.05	17.70	4.427	53.01
Control....	12/22– 1/12	6	8.030	1.895				23.58			

egg size it is only in the eggs of one of the four birds for which sufficient data were obtained (♀ 907, table 1) that the results can be interpreted as indicating an increase in total amount of inorganic matter under the calcium dosage.

On the other hand, several averages given indicate that the *percentage* of ash in the dry shell substance is higher in shells derived from the

dosage periods. Seven group comparisons are possible—three of groups from heavy dosage compared with control (table 1, last column) and four of groups from smaller dosage compared with control. Of these seven groups only one (from smaller dosage, ♀ K459, table 3) gives a lower percentage of ash under dosage. The summary given in the

TABLE 2

Effects upon various egg-structures obtained by feeding calcium lactate and calcium lactophosphate to female ring-doves (no. A120, upper division; E317, middle division; A843 lower division of table)

TREATMENT	DATE OF EGGS	NUMBER OF EGGS	AVERAGE WEIGHT IN GRAMS						PERCENTAGE RELATIONS OF PARTS		
			Egg	Yolk	Albumen	Shell			Yolk to egg	Shell to yolk	Shell to egg
						Moist	Dry	Ash			
Control..	11/ 2–11/12	4	8.677	1.961	6.268	0.4528	0.4478		22.53	23.19	5.174
Smaller dosage.	12 /4–12/6	2	8.444	1.936	6.060	0.4521	0.4477		22.94	23.15	5.301
Heavy dosage.	12/18–12/20	2	8.316	1.829	6.036	0.4563	0.4518		21.98	24.78	5.436
Control..	12/30– 1/19	6	8.613	1.896					21.96		
Control..	11/ 2–11/26	4	8.978	1.682	6.878	0.4238	0.4187	0.2282*	18.72	25.00	4.672
Smaller dosage.	11/26–12/9	1	8.718	1.773	6.534	0.4157	0.4108		20.33	23.17	4.712
Heavy dosage.	12/10–12/20	1	8.886	1.669	6.841	0.3807	0.3756		18.78	22.27	4.286
Control..	12/28– 1/31	5	8.698	1.603					18.44		
Control..	11/ 1–11/15	4	8.873	2.239	6.224	0.4152	0.4102	0.2153[1]	25.23	18.34	4.627
Smaller dosage.	11/30–12/13	3	8.576	2.178	5.981	0.4219	0.4165		25.39	19.22	4.855
Control..	12/23– 1/5	4	8.711	2.113					24.26		

* For a single egg whose weight was 8.640 grams; shell (dry), 0.4271 gram; and whose ash was 53.43 per cent of the shell.

[1] For a single egg whose weight was 9.022 grams; shell (dry), 0.3981 gram; and whose ash was 54.08 per cent of the shell.

last column of table 4 indicates that the dry weight of shells produced under the calcium dosage yields 53.7 per cent of ash as compared with 52.7 per cent from normal shells. However, inspection of the last column of table 3 will show that three birds—not included in any averages because none of the shells from the dosage period was ashed—have

quite high percentages for the control period. Also, it seems that low percentages in control usually gave higher percentages under dosage, and vice versa. The seeming difference in ash percentage just noted is, therefore, probably insignificant.

TABLE 3

Effects upon various egg-structures obtained by feeding calcium lactate and calcium lactophosphate to female ring-doves (no. K459, upper division; 152 and A798 next divisions; 48, lower divisions of table)

TREATMENT	DATE OF EGGS	NUMBER OF EGGS	AVERAGE WEIGHT IN GRAMS					PERCENTAGE RELATIONS OF PARTS			
			Egg	Yolk	Albumen	Shell (dry)	Ash	Yolk to egg	Shell to yolk	Shell to egg	Ash to shell
Control..	11/ 6–11/ 8	2	7.548	1.700	5.496	0.3530	0.1891	22.51	20.87	4.695	53.57
Smaller dosage.	11/17–12/20	6	7.721	1.817	5.552	0.3524	0.1856	23.47	19.60	4.573	52.83
Control..	12/29– 1/21	6	7.982	1.870				23.35			
Control..	11/12–11/26	4	3.325	1.986	5.926	0.4132	0.2196	23.81	20.94	4.966	53.68
Smaller dosage.	12/11–12/13	2	8.145	2.000	5.742	0.4038		24.55	20.23	4.959	
Control..	12/25– 1/7	4	8.245	1.859				22.53			
Control..	10/30–11/11	4	10.243	2.434	7.333	0.4770	0.2492*	23.69	19.90	4.681	53.85*
Smaller dosage.	11/27–11/29	2	9.809	2.306	7.030	0.4733		23.46	20.58	4.824	
Heavy dosage.	12/28– 1/27	6	10.008	2.300				22.96			
Control..	11/12–11/28	3	8.367	2.050	5.858	0.4586	0.2562[1]	24.50	22.45	5.481	54.38[1]
Lightest dosage.	12/ 6–12/8	2	7.822	1.969	5.451	0.4028		25.15	20.51	5.156	
Control..	12/29– 2/2	5	7.711	1.821				23.04			

* Average for two eggs whose weight was 10.410 grams; shell (dry), 0.4627 gram.
[1] For a single egg whose weight was 8.561 grams; shell (dry), 0.4711 gram.

A decrease in egg size during the calcium feeding has already been noted. It remains to locate the part or parts of the egg which undergo this reduction. The size of the enclosed yolk is ordinarily and normally the chief, though indirect, determining factor in ultimate egg weight. The data show that there was perhaps a slight decrease in yolk size during the dosage period. But an examination of the average weights

of "yolk" and "albumen" in table 4 clearly shows that, even in cases where yolk weight was fully maintained in dosage periods, the albumen

TABLE 4

Summaries (weighted averages throughout) of effects of smaller and heavier dosage of calcium lactate and calcium lactophosphate on the various egg-structures of ring-doves (A622, 907, A347, E317 and A120 in upper division of table; all included in lower division of table)

TREATMENT	NUMBER OF EGGS	AVERAGE WEIGHT IN GRAMS					PERCENTAGE RELATIONS OF PARTS			
		Egg	Yolk	Albumen	Shell (dry)	Ash	Yolk to egg	Shell to yolk	Shell to egg	Ash to shell
For five birds (smaller and heavy dosage)										
Control	20	8.762	1.947	6.3884	0.4323		22.23	22.43	4.928	
Smaller dosage	15	8.474	2.008	6.0609	0.4164		23.60	20.92	4.904	
Heavy dosage	14	8.297	1.983	5.8960	0.4224		24.13	21.11	5.007	
Control	26	8.394	1.941				22.66			
Control[1]	12	8.718	2.031	6.265	0.4301	0.2268	23.30	21.18	4.933	52.73
Smaller dosage[1]	12	8.460	2.027	6.022	0.4117	0.2211	23.96	20.31	4.866	53.70
Heavy dosage[1]	11	8.238	2.042	5.785	0.4123	0.2214	24.79	20.29	5.005	53.70
For ten birds (smaller dosage only)										
Control	37	8.785	2.025	6.331	0.4300		23.05	21.23	4.895	
Smaller dosage	30	8.358	1.998	5.889	0.4060		23.91	20.32	4.858	
Control	51	8.483	1.927				22.72			
Control[2]	14	8.540	1.983	6.155	0.4191	0.2214	23.22	21.13	4.907	52.45
Smaller dosage[2]	18	8.214	1.980	5.865	0.3918	0.2094	24.11	19.79	4.770	53.45
Control[2]	21	8.143	1.960				24.07			

[1] This part of summary—including *ash* determinations for the three series—can be given for only three (A622, 907 and A347) of the five females.

[2] This part of summary—including *ash* determinations—can be given for only four (A347, K459, 907 and A622) of the ten females.

fell plainly below the control and that practically the whole of the loss of egg weight is due to the presence of distinctly less albumen. The slight reduction in average yolk size is almost certainly attributable to

another circumstance.[1] In our opinion the marked reduction of albumen secreted in the dosage periods is, in one way or another, attributable to the addition of the calcium salts to the diet.

The ten birds that have been described were not birds which had produced, nor were then producing, obviously thin-shelled eggs. However, the details of our data show that the control eggs of any individual bird were unequally provided with total shell-substance and with inorganic matter. In those cases therefore it would seem that a larger supply of the necessary inorganic matter used in shell formation might assist in raising the slightly lighter shells to the level of the heavier ones. In addition, two females which were producing soft, thin or obviously defective shells were dosed (0.226 gram daily) for a period in order to learn whether normal shells might be thus produced. Neither of these birds produced any eggs during 3 weeks of dosage; their dosage was therefore discontinued. In two other tests of similar females, with dosage extended to 1–3 months, the production of eggs with soft or thin shells and early-dying embryos was continued.

DISCUSSION

As results of this study two points are fairly clear. First, that the amount of inorganic substance laid down in the egg shells was practically unchanged by the extra calcium intake. The dry weight of shells produced during dosage seems slightly under normal weight, while an equal or insignificantly higher percentage of ash is present in these slightly lighter shells. Second, the unexpected circumstance that the extra calcium feeding resulted in a reduced secretion of albumen.

Disturbances incident to the catching and dosage of our birds, and above all a conceivable degree of nausea from the calcium salt in the crop, may have tended toward a *reduced* calcium assimilation. This inference could be drawn from the work of Meigs, Blatherwick and Cary cited above. Also, if the salt produced any lack of appetite—which would have escaped observation—the normal source of calcium may have been thus reduced. These assumptions could be considered in connection with the failure of our calcium feeding to increase the amount of shell-substance if they could better meet the difficulties in

[1] Females A798 and 48 failed to produce eggs continuously under dosage and therefore obtained a period of "reproductive rest." Such a period of rest has been previously found to be normally followed by yolks of smaller size. Female E317 obtained partial periods of rest and produced only one *pair* of eggs, and five *unpaired* eggs after the early "control" data.

explaining the observed changes in the albumen. Disturbances incident to the handling of the birds certainly can not account for the decreased production of albumen, since wholly similar birds were caught and blank-dosed in connection with an earlier study and it was learned that no change is thus produced. That nausea or under-feeding could produce a decreased secretion of albumen is perhaps not improbable. But the average body weights of the birds remained practically constant under dosage, and this fact is difficult to reconcile with any under-feeding during dosage. Again, the rate of egg-production was unchanged.

Our own observations on the condition of our experimental animals, and the results of similar earlier investigations, permit us to consider it highly probable that these doves absorbed and excreted more calcium while under dosage; our data demonstrate nevertheless that little or no change was produced in the amount of material deposited in the shell. That the maximum of shell production was not attained, neither in the control nor in the calcium dosage periods, is plainly evident from the details obtained for individual eggs and from other observations and experience as well. Our results therefore support Buehner and Martin's conclusion that the lack of calcium is not the fundamental cause of the production of thin-shelled eggs. Their conclusion was based upon data derived from under-feeding of inorganic calcium to fowls; our conclusion is based upon extra feeding of soluble calcium to ring-doves.

Wheeler (13) found, somewhat earlier than Buckner and Martin, that a long continuance of a diet low in calcium results in the production by fowls of some eggs with thin shells; and that calcium is earlier freely removed from the bones for shell formation. Wheeler also made the further interesting observation that strontium can very largely replace calcium in both the shell and bones of fowls and ducks, though magnesium is incapable of doing so.

On the puzzling result concerning the reduced albumen secretion observed by us we can offer only the following suggestion. If a sufficient excess of calcium were present in the circulation, the well-known action of calcium might have effected a depression of the nerve, muscle or gland cells of the albumen-secreting gland. Any muscular effect which would permit or induce a more rapid transit of the egg through the albumen-secreting part of the oviduct (the total time of passage through the entire oviduct was certainly not modified), or a diminished activity of the albumen secreting cells, or a general reduction of the body metabo-

lism, could account for the decrease actually observed. It seems less probable that the time spent in the albumen-secreting gland was shortened than that the normal rate of activity of the gland was reduced.

SUMMARY

Calcium lactate and calcium lactophosphate were added in various amounts to the normal diet of freely laying ring-doves. The possible quantitative changes in the shell and in other gross egg constituents were measured.

The amount of dry shell-substance was not increased, but perhaps slightly diminished, under the extra calcium feeding. The percentage of inorganic matter in the dry shell-substance was probably unchanged.

The amount of albumen secreted under extra calcium feeding was measurably decreased.

No measurable change in the rate of reproduction (ovulation) occurred in the treated birds.

The production of inadequate shells, or of thin-shelled eggs, which is associated with the early death of many bird embryos is probably not caused by an inadequate calcium supply in the food. The feeding of organic calcium salts to female ring-doves failed appreciably to strengthen their shells.

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STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

XII. The Relation of Nerve Stimuli to Oviducal Secretions as Indicated by Effects of Atropine and Other Alkaloids

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The oviduct of birds contains three glands whose markedly intermittent activity gives rise to three different and highly specialized products—egg-albumen, shell membrane and egg shell. The relation of nerve stimuli to this intermittent glandular activity is apparently quite unknown. It is well known, however, that the presence or placement of solid or semi-solid objects within the upper oviduct usually, but not invariably, results in the secretion of albumen, membrane and shell. Such an introduced object may be an egg yolk or any one of many substitutes such as feathers or dirt caught up by the everted oviduct, an amber bead (1), a plug of wood or rubber (2), a mass of feces or of agar (3). Observations of this sort give support to the view that these glandular secretions occur in response to contact, mechanical or pressure stimuli necessarily connected with the presence of the foreign object; but whether local reflexes are also directly involved is a matter wholly in doubt.

Of much importance in this situation is the fact that introduced foreign bodies can induce these secretions only in an oviduct which is in an active or functional state; and that this functional state is attained as a result of, or in close association with, processes occurring in the ovary. An internal secretion is therefore apparently involved in this preliminary preparation or stimulation of the gland. Whether this same hypothetical internal secretion which prepares the gland for activity is also an active or effective agent in inducing the actual secretion, once a contact stimulus is supplied, is a question really unanswered by such data as are now available for the bird.

The possibility of nervous control of these secretions certainly exists, though the necessary specific facts are at present lacking. Unfortunately, we have been unable to find any definite anatomic knowledge of the innervation of the glands of the avian oviduct; and physiological or pharmacological studies on these nerves of the bird seem never to have been attempted. In the absence of definite information one may nevertheless begin an investigation upon the following assumptions: First, that the innervation of the oviduct is similar to that of the homologous organ—the uterus—of mammals. Even among amphibians Langley and Orbelli (4) found "that in its general features the plan of the sympathetic innervation of the viscera of the frog is the same as that of the mammal." Second, that the highly glandular part as well as the muscular part may have received parasympathetic and sympathetic innervation in birds. Third, that the action of atropine and other alkaloids is likely to be rather similar in the two organs. If these assumptions approximately represent the facts we may reasonably hope to decrease or to increase the oviducal secretions of the dove by means of proper doses of those alkaloids which have been found markedly to affect secretions under the control of autonomic nerves in mammals; one may, for example, expect to diminish the secretions by atropine.

It is understood, however, that a decreased secretion of albumen or of shell would not be a necessary result of the depressant action of atropine since it is known that parasympathetic depression or stimulation does not respectively decrease or increase the output of all glands having this innervation. And until now apparently we have little or no data by which thus to classify the oviducal glands of the bird. Again, there is the possibility that any observed effect on the amount of secretion under the drug may be the result of the action of the latter on the gland cells themselves. In view of these and still other uncertainties we shall here confine ourselves largely to an objective description of our results. When further facts shall have been accumulated a fuller interpretation may be possible. Also, other investigators may be in possession of information which has not been available to us. Since, however, we have made use of drugs—atropine, cocaine, nicotine, pilocarpine—which are reputed to have such characteristic action on the autonomic nerves of the mammal it seems to us highly probable that our results throw some light upon the extent to which the secretion of albumen and shell are dependent upon nerve stimuli. In any case our data supply a measure of the effect or lack of effect of particular dosages of these drugs upon these secretions of the oviduct.

Our special reason for undertaking this study was our need of the results of this inquiry in a series of studies whose object is to learn why the eggs of some birds are habitually or sporadically provided with thin or inadequate shells. That general problem has been discussed earlier by one of us (5). In the immediately preceding paper of this series (6) it was found that an increased intake of soluble calcium salts has very little effect upon the amount of calcium laid down in the shell. The question next arose: Is the secretion of the shell under the control of nerves? Are the egg shells of birds sometimes inadequate because of nervous deficiency or derangement? The present study has been carried out with particular reference to a decision of this point. If, however, the secretion of shell and albumen are found to be not really under nervous control the question of the possible mechanisms which play a part in regulating the intermittent activity of those glands is placed one step nearer solution,—since, in that case, direct contact stimuli and the influence of an internal secretion seem the only other alternatives.

Materials and methods. Eight female ring-doves (blond and white ring-dove hybrids) which were producing pairs of eggs (clutches) at intervals of 6 to 8 days were selected for study. These birds had been previously fed calcium lactate during a period of a month or more, as reported elsewhere (6). A period of 40 to 60 days elapsed after the lactate feeding before the treatment here recorded was begun. These birds had an average body weight of 169 grams at the beginning and 165 grams at the close of atropine administration. Body weight after the short cocaine, nicotine and pilocarpine dosage was not determined.

Atropine sulfate (Parke, Davis Co.) was administered in four dosages. The lightest dosage was of 0.002 grain, once daily, tested on a single bird (♀48, table 3). Other dosages were: 0.002 grain (7 birds), 0.005 grain (7 birds), 0.01 grain (2 birds); all given twice daily at about 9:00 a. m. and 7:00 p. m. The heavier dosages were given to birds which had earlier been given lighter dosage.[1] Willburg (7) determined the lethal dosage of subcutaneously injected atropine sulfate in a number of animals; for the dove he found this to be 0.2 to 0.25 gram per kilogram body weight. The dove was found 113 times more resistant to the drug than is man. In our administration of the drug the relatively tame birds were caught and tablets of appropriate size dropped far back into the throat. Birds were frequently watched for regurgitation; none was ever noted. Four birds (table 4) were given 0.2 and 0.4 grain atropine thrice daily.

[1] For the extremely heavy dosage of atropine and pilocarpine (table 4) another group of eight females was used. These birds had not previously been treated with any drug.

The only supply of nicotine (Merck) available to us had been purchased nearly six years before using. It had been kept unopened in the dark and its strength was tested as follows: A dove of 175 grams body weight, injected subcutaneously with 1.67 mgm. died in 1 to 1½ minutes. A similar dove given 0.67 mgm. vomited, showed slight diarrhea, and distinctly heavier respiration. Within ½ hour the dosage to this bird was increased by 0.57 mgm. The above symptoms (except diarrhea) increased, eyelid movements became abnormal and standing position unsteady. A third dove survived a dosage of 1.0 mgm. but showed many signs of collapse. All nicotine solutions were made up in water each 3 days and kept in the dark. The strength of the solutions was made such as to require subcutaneous injections of only ½ cc.

Cocaine (Boehringer) was converted by us into the hydrochloride and subcutaneously injected in aqueous solution.

Pilocarpine hydrochloride (U. S. P.) was given per os as was the atropine.

None of the above drugs was given continuously, but solely with reference to the time of egg-laying. These doves lay two eggs in a clutch, the two being laid 40 hours apart. The first of the pair is laid at very nearly 5:00 p.m.; the second at nearly 9:00 a.m. of the second morning thereafter. When the eggs are removed from the nest immediately after laying, the first egg of a next following pair may usually be expected 6 or 7 days thereafter. The egg requires about 45 hours for passage down the oviduct. An egg laid 6 days after the last of the previous pair would therefore leave the ovary and begin to receive the oviducal secretions about 4 days after the laying of the last preceding egg.

Since our purpose was not to study effects of the dosage upon the work of the ovary (yolk size),[2] and in order to avoid the probable cessation of egg production incident to continuous dosage, atropine was given only from the morning of the 3rd day after the laying of the last of the preceding pair of eggs. The albumen is secreted during the first 15 of the 45 hours spent by the egg in the oviduct; the formation of the shell occurs during the last 30 hours. The administration of nicotine and of cocaine was so timed as to affect the secretion of the shell only, of the first egg of the prospective pair; this, however, necessarily subjected the albumen secretion of the second egg of the pair to the action of the drug.

The eggs and shells were prepared in the following manner: Eggs were obtained 10 minutes to 3 hours after laying; they were weighed, steamed for 8 minutes, cooled in tap water for 4 minutes and the shell removed piece by piece with small curved-tip forceps. The weights of the solid coagulated yolk and of the moist (nearly air dry) shell were next obtained. The sum of these two weights subtracted from the total egg weight gave the weight of the albumen (including shell membrane). The albumen cannot be weighed directly on account of evaporation during removal of the shell.

Since nearly all of the inorganic material of the shell is in the form of calcium carbonate[3]—with magnesium carbonate and earthy phosphates present in very

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[2] Unavoidably, however, the *yolk* of the first of the pair was subjected during its last 1 to 2 days in the ovary to the action of the drug; while the *yolk* of the second of the pair was thus affected during the last 3 to 4 days of its growth in the ovary.

[3] Buckner and Martin (8) found in shells of fowls' eggs that the calcium calculated as CaO, is equal to about 98.0 per cent of the ash.

small amounts and representing practically all of the remainder—it was found practicable and desirable, because of the numerous samples, to make volumetric determinations of the total of these alkaline earths. The egg shell was dried in a weighing bottle for 12 hours at 105°C. It was found that the organic matrix of the shell prevented ready solubility in weak acid. Since the nature of the procedure made it inadvisable to use a strong acid the dry shell was transferred to a porcelain crucible and ignited until the small amount of organic material had been removed and then over a heavy flame until all carbonate was converted into oxide. The crucible and ash were then placed in a beaker and the ash dissolved in 100 cc. N/10 HCl. By using 100 cc. there was an excess of 10 to 40 cc. which was determined by titration with N/10 NaOH. Methyl orange was used as indicator. The values for total bases thus obtained are considered as wholly calcium and calculated in the tables as per cent $CaCO_3$ in dry shell.

Presentation of data. For obvious reasons it seemed best to study the effects of the drugs chiefly—though not exclusively—on birds whose secretions were normal and to place chief reliance upon a decreased secretion under atropine.

The details of data for individual egg-shells and albumens as obtained from one bird are fully given in table 1. In order to economize space other records (tables 2 and 3) are given in summary only. Reasons for inclusion of yolk size and other data in these tables are given in a preceding paper (5), and the discussion given there of the relations which normally obtain between yolk size on the one hand and the volume of the oviducal secretions on the other are necessary to a proper estimate of the present data.

Atropine. The summary given at the bottom of table 3 makes it clear that under atropine dosage the amount of albumen secreted is slightly though certainly reduced. The reduction is not more than 2 or 3 per cent. Analyses of this albumen show (table 5) that it was nearly or quite normal in respect to the relative proportions of water, alcohol-soluble and alcohol-insoluble constituents.

The amount of shell material secreted under atropine was either unaffected or but slightly affected. In this evaluation the smaller amount of egg surface presented by the eggs obtained under atropine is of some importance as shown by reference to the figures for "percentage relations of shell to egg" (tables 1 to 3). The percentage of alkaline earths present in the shell-ash was possibly slightly reduced under atropine. The difference involved is of questionable value.

The two birds (table 1; and ♀907, table 2) which each received three different degrees of atropine dosage do not plainly show a greater effect of the dosage with 0.02 grain daily than with 0.004 grain daily. Yolk size was unaffected by the short part of its growth period during

TABLE 1

Effects of atropine and of nicotine on the oviducal secretion of ring-dove A120

TREATMENT	DATE OF EGG	AVERAGE WEIGHT IN GRAMS					PER CENT OF BASES IN SHELL ASH (AS $CaCO_3$)	PERCENTAGE RELATIONS OF PARTS			
		Egg	Yolk	Albumen	Shell			Yolk to egg	Yolk to albumen	Shell to egg	Shell to yolk
					Moist	Dry					
Control	1/26	8.581	2.018	6.119	0.4440	0.4361	95.51	23.52	32.98	5.082	21.61
	1/28	8.761	2.230	6.133	0.3982	0.3918	95.13	25.45	36.36	4.472	17.57
	2/4	8.771	1.954	6.382	0.4350	0.4285	95.81	22.28	30.62	4.885	21.93
	2/13	9.409	2.077	6.841	0.4913	0.4813	95.40	22.08	30.36	5.115	23.17
	Average.	*8.881*	*2.070*	*6.369*	*0.4422*	*0.4344*	*95.46*	*23.33*	*32.58*	*4.889*	*21.07*
Atropine	2/20*	8.250	1.892	5.990	0.3679	0.3638	94.60	22.93	31.59	4.410	19.23
	3/1	8.194	1.885	5.854	0.4552	0.4464	95.44	23.00	32.20	5.448	23.68
	3/3	8.826	2.150	6.231	0.4453	0.4320	95.53	24.36	34.50	4.895	20.09
	Average.	*8.423*	*1.976*	*6.025*	*0.4228*	*0.4141*	*95.19*	*23.43*	*32.76*	*4.918*	*21.00*
Atropine	3/10[1]	8.925	2.019	6.438	0.4678	0.4556	94.63	22.62	31.36	5.105	22.57
	3/12	8.844	2.090	6.315	0.4385	0.4241	95.63	23.63	33.10	4.795	20.29
	3/19	8.793	1.951	6.371	0.4710	0.4535	95.30	22.19	29.24	5.158	23.25
	3/21	9.071	2.141	6.493	0.4372	0.4277	94.32	23.60	32.97	4.715	19.98
	Average.	*8.908*	*2.050*	*6.404*	*0.4536*	*0.4402*	*94.97*	*23.01*	*31.67*	*4.943*	*21.53*
Atropine	3/28[2]	8.659	1.901	6.308	0.4495	0.4412	95.27	21.95	30.14	5.095	23.21
	3/30	8.875	2.089	6.366	0.4201	0.4089	95.40	23.54	32.81	4.607	19.57
	4/5	7.991	1.609	5.952	0.4297	0.4181	96.37	20.14	27.03	5.232	25.99
	4/7	8.820	1.942	6.449	0.4287	0.4134	96.00	22.02	30.11	4.687	21.29
	Average.	*8.586*	*1.885*	*6.269*	*0.4320*	*0.4204*	*95.76*	*21.91*	*30.02*	*4.905*	*22.52*
Control	4/13	8.558	1.741	6.341	0.4761	0.4644	95.81	20.34	27.39	5.427	26.67
	4/15	9.202	2.092	6.680	0.4304	0.4170	95.59	22.73	31.32	4.532	19.93
	4/21	8.430	1.783	6.185	0.4617	0.4513	95.30	21.15	29.50	5.354	25.31
	4/23	8.874	2.035	6.401	0.4379	0.4206	96.20	22.93	31.79	4.740	20.67
	Average.	*8.766*	*1.913*	*6.402*	*0.4515*	*0.4383*	*95.72*	*21.79*	*30.00*	*5.013*	*23.14*
Nicotine	5/9[3]	7.987	1.659	5.899	0.4247	0.4120	93.68	20.77	28.12	5.158	24.83
	5/17[4]	8.516	1.729	6.275	0.5115	0.4954	95.72	20.30	27.55	5.817	28.65
	5/19	8.583	1.805	6.312	0.4658	0.4414	96.21	21.03	28.60	5.143	24.45
	Average.	*8.362*	*1.731*	*6.162*	*0.4673*	*0.4496*	*95.20*	*20.70*	*28.09*	*5.373*	*25.98*
Control	5/26	8.726	1.936					22.19			
	5/28	9.201	2.129					23.14			
	6/3	8.723	1.898					21.76			
	6/5	8.766	2.035					23.21			
	Average.	*8.854*	*2.000*					*22.58*			

* Atropine dosage (0.002 grain twice daily) began on 2/16.

[1] Heavy dosage (0.005 grain twice daily) from 3/6 to 3/21.

[2] Extra heavy dosage (0.01 grain twice daily) from 3/24 to 4/6

[3] Injections of nicotine (0.19 mgm. once daily, at 12:45 p. m.—given from 5/8 to 5/10.

[4] Injections of nicotine (0.19 mgm. each); 1 on 5/16, 2 on 5/17, 2 on 5/18.

TABLE 2

Effects of atropine, cocaine and nicotine on the oviducal secretions of ring-doves 907, A798, A843, 152.

(Birds in this order from top of table)

TREATMENT	DATA ON EGGS		AVERAGE WEIGHT IN GRAMS				PER CENT OF BASIS IN SHELL ASH ($CaCO_3$)	PERCENTAGE RELATIONS OF PARTS			
	Number	Date	Egg	Yolk	Albumen	Shell (dry)		Yolk to egg	Yolk to albumen	Shell to egg	Shell to yolk
Control....	6	1/24–2/12	9.755	2.051	7.204	0.4567	95.51	21.01	29.05	4.681	22.34
Atropine*..	4	2/19–3/2	9.356	2.067	6.835	0.4465	95.41	22.10	30.26	4.774	21.61
Atropine[1]..	4	3/10–3/31	8.765	2.022	6.284	0.4523	95.29	23.06	30.20	5.157	22.43
Atropine[2]..	4	3/28–4/8	8.698	2.015	6.244	0.4322	95.39	23.16	32.28	4.972	21.47
Control....	4	4/14–4/25	8.919	2.036	6.432	0.4401	95.69	22.77	31.75	4.935	21.73
Nicotine[3]...	1	5/2	9.009	1.958	6.633[3]	0.4096	95.61	21.73	29.52[3]	4.547	20.91
Control....	3	5/16–5/24	8.031	1.939				24.23			
Control....	4	1/25–2/9	9.930	2.290	7.185	0.4481	95.74	23.04	31.85	4.521	19.63
Atropine*..	2	2/16–2/18	10.136	2.407	7.322	0.4031	95.38	23.69	32.78	4.016	17.05
Atropine[1]..	4	3/24–4/5	9.330	2.040	6.821	0.4612	95.62	21.86	29.91	4.949	22.58
Control....	6	4/13–5/3	9.826	2.196	7.201	0.4185	95.07	22.32	30.47	4.293	19.28
Nicotine[4]..	4	5/12–5/23	9.491	2.095	6.909	0.3980	95.36	22.05	30.00	4.216	19.19
Control....	4	5/30–6/10	9.881	2.144				21.71			
Control....	4	1/27–2/9	8.694	2.082	6.193	0.4081	95.98	23.91	33.62	4.699	19.70
Atropine*..	4	2/19–3/3	8.728	2.244	6.084	0.3916	95.62	25.71	36.88	4.490	17.48
Atropine[1]..	4	3/11–3/24	8.740	2.144	6.218	0.3698	94.95	24.51	34.46	4.238	17.35
Control....	4	3/31–4/11	9.012	2.162	6.464	0.3734	95.54	23.99	33.45	4.144	17.29
Cocaine[5]...	5	4/18–5/7	8.681	2.089	6.239	0.3477	95.25	24.01	33.59	4.001	16.64
Nicotine[6]..	4	5/15–5/26	8.559	2.126	6.028	0.3832	95.91	24.86	35.33	4.483	18.05
Control....	4	6/ 2–6/13	8.919	2.168				24.28			
Control....	4	1/28–2/10	8.111	1.869	5.859	0.3761	95.75	23.00	31.90	4.635	20.17
Atropine*..	4	2/17–2/29	7.848	1.822	5.662	0.3573	94.90	23.20	32.18	4.550	19.67
Atropine[1]..	4	3/ 7–3/18	7.841	1.869	5.606	0.3492	95.28	23.86	33.34	4.592	19.31
Control[7]...	4	3/25–4/4	7.830	1.768	5.707[7]	0.3480	95.39	22.79	30.98	4.494	19.77
Cocaine[8]...	6	4/18–5/9	7.600	1.765	5.492	0.3341	95.74	23.29	32.33	4.397	18.98
Control	2	5/16–5/18	7.993	1.735	5 889	0.3541	96.25	21.70	29.45	4.434	20.45

*Atropine dosage of 0.002 grain twice daily.

[1] The dosage was 0.005 grain twice daily.

[2] Atropine dosage of 0.01 grain twice daily began 3/24, last dose given 4/7 p. m.

[3] Dosed with nicotine (0.2 mgm.) 5/1 p. m. and 5/2 a. m.; albumen secretion already complete.

[4] For the first pair of eggs the dosage was 0.19 mgm. of nicotine injected once daily from 3 days before first of pair was laid; for the second pair of eggs the dosage was 0.38 mgm. once daily beginning at 12:45 p. m. on the date the first of the pair was laid.

[5] Injections of 0.6 mgm. cocaine thrice daily for first pair, beginning 1 day before laying of first egg; for next (single) egg, 0.5 mgm. thrice daily; for last pair of eggs the same dosage beginning 2 days before laying first of pair.

[6] Injections of 0.19 mgm. nicotine once daily for first pair; of 0.38 mgm. once daily for second pair; first injection 2 days before first egg in both cases.

[7] Atropine dosage discontinued on 3/23—only 2 days before laying first egg of this control series.

[8] Three injections daily of 0.6 mgm. cocaine hydrochloride for first pair of eggs; 0.5 mgm. for last four eggs. For two pairs injections began 1 day before first egg; for third pair, 3 days earlier.

TABLE 3

Summary of effects of atropine on the oviducal secretion of ring-doves A622, 48, E317. (In this order from top of table)

TREATMENT	DATA ON EGGS		AVERAGE WEIGHT IN GRAMS				PER CENT OF BASES IN SHELL ASH (AS $CaCO_3$)	PERCENTAGE RELATIONS OF PARTS			
	Number	Date	Egg	Yolk	Albumen	Shell (dry)		Yolk to egg	Yolk to albumen	Shell to egg	Shell to yolk
Control...	2	1/28–2/5	7.061	2.086	4.616	0.3533	95.23	29.54	45.19	5.001	16.92
Atropine*.	4	2/13–2/28	6.801	2.161	4.282	0.3528	95.47	31.87	50.47	5.184	16.33
Atropine[1].	5	3/7–3/23	6.677	2.088	4.232	0.3492	94.81	31.24	49.34	5.217	16.71
Control...	5	3/29–4/14	6.721	2.023	4.355	0.3331	95.29	30.10	46.45	4.963	16.49
Control...	3	1/31–2/8	7.960	1.877	5.643	0.4328	95.94	23.56	33.26	5.439	23.17
Atropine[2].	5	2/17–3/19	7.855	(1.874)	5.576	0.3936	95.69	23.84	33.60	5.042	21.21
Control...	5	3/25–4/15	8.201	2.151	5.677	0.3623	(94.80)	26.21	37.88	4.415	16.90
Control...	4	1/29–2/10	8.477	1.620	6.415	0.4072	95.74	19.09	25.25	4.805	25.20
Atropine*.	2	2/18–2/28	8.313	1.715	6.222	0.3700	95.37	20.63	27.56	4.448	21.59
Atropine[1].	3	3/7–3/17	7.861	1.488	6.009	0.3557	94.74	18.93	24.76	4.513	23.92
Control..		3/26[3]	7.823	1.695	5.729	0.3902	Lost	21.67	29.59	4.988	23.02
		4/3[3]	broken			0.4245	93.69				
		4/9[3]	7.508	1.431	5.726	0.3449	91.48	19.06	24.99	4.594	24.10
	5–6	4/14	6.683	1.438	4.955	0.2845	94.49	21.52	29.02	4.257	19.71
		4/24[3]	6.992	1.462	5.154	0.3665	94.57	20.91	28.36	5.242	25.00
		4/30[3]	7.370	1.411	5.547	0.4008	94.89	19.15	25.44	5.438	28.41
		Average.	*7.275*	*1.487*	*5.422*	*0.3686*	*93.82*	*20.46*	*27.43*	*4.904*	*24.05*
Summary[4]											
Control..	22		9.144	2.062	6.601	0.4276	95.67	22.69	31.55	4.685	20.74
Atropine..	17		8.781	2.075	6.297	0.4018	95.29	23.63	33.01	4.591	19.54
Atropine..	20		8.717	2.025	6.267	0.4125	95.22	23.26	31.92	4.776	20.64
Control..	22		8.957	2.032	6.510	0.4050	95.44	22.69	31.25	4.550	20.06

* Atropine dosage of 0.002 grain twice daily.

[1] Atropine dosage increased to 0.005 grain (twice daily) from 3/3 a. m. to 3/23.

[2] Atropine dosage (0.002 grain *once* daily) began on 2/13; not dosed after 3/23.

[3] This egg abnormally delayed in lower oviduct.

[4] The three birds whose records are given immediately above on this table either ceased producing eggs (48), or laid only abnormal eggs (A622,E317), very soon after the above records. This summary therefore excludes these three records. Also the highest dosage of atropine cannot be represented here since this dosage was given to only two of the five birds included in the summary.

TABLE 4

The effects of pilocarpine (and very heavy atropine) dosage on the thickness of shells as measured by hourly rate of loss

NUMBER OF BIRD	DOSAGE		DATE OF EGGS	TIME ACTUALLY LAID (1STS = P.M.) (2NDS = A.M.)	TREATED EGGS		MEANS FOR CONTROL EGGS	
	Amount	Time before first egg			Weight	Rate of loss	Weight	Rate of loss
	grains	*hours*			*grams*	*mgm.*	*grams*	
P904	(0.04)	20	3/18	Before 5:45	8.11	3.6	8.69	3.3
			3/20	Before 7:45	9.17	5.9	9.69	7.0
P891	(0.04)	20	3/19	4:45–5:10	7.80	3.5	8.05	(12.6)[3]
			3/21	8:30–9:00	8.41	([3])	7.84	
P900	(0.02)*	0	3/18	Before 5:30	8.60	3.3	8.56	3.4
			3/20	10:00–10:45	9.89	4.0	9.86	5.5
A250	(0.02)*	20	3/19	4:45–5:05	8.08	3.3	8.24	3.5
			3/21	8:30–9:00	9.62	5.0	9.38	4.5
P839[1]	(0.04)*	0	3/19	At 4:40	6.65	3.3	6.85	3.6
			3/21	At 9:20	7.58	7.6	7.29	10.4
P692	(0.04)*	0	3/19	At 4:49	7.23	3.5	7.22	3.4
			3/21	9:30–10:00	8.05	5.1	8.19	4.4
P830	(0.06)	0	3/20	4:10–4:40	9.66	3.7	9.25	3.6
			3/22	Before 7:45	9.89	3.1	9.88	4.2
P853[1]	(0.06)	0	3/20	At 4:39	8.99	([3])		
			3/22	12:00–2:35	9.20	([3])		
P708	(0.04)[2]	0	3/25	Before 9:40	7.58	4.8	8.20	6.5
			3/27	Before 11:30	8.90	7.6	8.44	6.7
P755	(0.04)	0	3/25	Before 5:00	7.79	3.1	8.23	3.1
			3/27	Before 11:30	8.70	4.5	8.81	4.2
A193	(0.04)	20	3/26	4:40–5:10	10.31	4.6	10.61	4.6
P889	(0.04)	20	3/27	1:50–8:30	7.48	3.0	7.42	3.7
			3/29	At 9:40	7.91	3.5	7.55	5.0
P887	(0.04)	20	3/27	1:50–8:30	8.87	4.0	8.29	4.1
			3/29	Before 7:50	10.24	4.5	9.62	4.6

TABLE 4—*Concluded*

NUMBER OF BIRD	DOSAGE		DATE OF EGGS	TIME ACTUALLY LAID (1STS = A.M.) (2NDS = P.M.)	TREATED EGGS		MEANS FOR CONTROL EGGS	
	Amount	Time before first egg			Weight	Rate of loss	Weight	Rate of loss
	grains	*hours*			*grams*	*mgm.*	*grams*	
P659	(0.02)	44	3/31	Before 4:50	8.21	3.2	8.46	3.2
			4/2	Before 8:25	8.94	3.5	8.87	3.5
907	(0.01)	20	4/2	At 5:37	9.25	4.0	9.31	3.3
			4/4	9:30–9:45	9.93	4.0	9.79	4.9
A798	(0.02)	98	4/2	At 5:56	8.85	3.4	8.95	4.8
			4/4	9:00–9:15	10.17	2.3	11.03	5.7
A843	(0.02)	164	4/5	At 5:10	8.25	3.2	8.17	4.1
			4/7	9:30–10:00	Broken			
P887	(0.02)	284	4/7	4:40–4:50	8.58	3.8	8.29	4.1
			4/9	Before 9:00	9.67	4.3	9.62	4.6
P889	(0.02)	356	4/10	At 4:35	7.09	3.0	7.42	3.7
			4/12	10:00–11:00	8.31	3.6	7.55	5.0
P659	(0.02)	284	4/10	At 4:56	7.84	3.7	8.46	3.2
			4/12	At 7:53	8.53	3.7	8.87	3.5
907	(0.01)	212	4/10	At 5:36	9.04	3.3	9.31	3.3
			4/12	At 8:48	9.79	4.1	9.79	4.9
A798	(0.02)	332	4/12	5:10–6:50	8.74	3.7	8.95	4.8
			4/14	9:30–9:45	9.72	5.7	11.03	5.7

* These four birds were dosed (three times daily) with atropine sulfate.

[1] This bird visibly much affected by dosage. First egg in life for no. P853.

[2] Dosage above this point in table was three times daily; including and below this point all dosage was twice daily.

[3] Shell thin and soon crushed.

which it was subjected to atropine. A dosage of 0.04 grain thrice daily brought one of two birds near to collapse, but neither this dosage nor one-half of this amount affected considerably or measurably the amount of shell material secreted by four birds as this was measured by the hourly rate of loss weight in table 4.

Cocaine. The secretion of albumen (last two birds of table 2) under cocaine was probably slightly decreased. The amount of shell material

apparently decreased by about 5 per cent. The percentage of bases present in the shell ash was little if at all affected; possibly it was slightly decreased but there is even less evidence of this than there was in the case of atropine.

Nicotine. Twelve eggs were obtained from four birds treated with nicotine (table 1, and first three birds of table 2). In only about one-half of these was the nicotine given before the secretion of albumen was completed. This fact and other special circumstances concerned in the figures obtained make it doubtful whether the data show an

TABLE 5

Analyses of albumen secreted under atropine dosage (0.005 grain, twice daily) and control

	NUMBER OF ANALYSIS	WEIGHT	PERCENTAGE (MOIST WEIGHT)		
			Alcohol insoluble	Alcohol soluble	H_2O
		grams			
Control	1	6.341	7.67	1.12	91.21
	2	6.417	8.37	1.26	90.37
	3	6.438	8.28	1.06	90.66
	4	6.692	7.12	1.08	91.80
	5	5.161	7.20	1.26	91.54
	6	6.340	7.39	1.10	91.51
	Average...	*6.232*	*7.67*	*1.15*	*91.18*
Atropine	7	6.204	8.01	1.25	90.74
	8	6.493	7.89	1.14	90.97
	9	6.371	8.28	1.22	90.50
	10	6.078	7.94	1.23	90.83
	11	5.694	8.28	1.10	90.62
	12	6.209	7.45	1.29	91.26
	Average....	*6.175*	*7.98*	*1.21*	*90.82*

effect, or indeed whether adequate opportunity was offered to produce an effect, on the secretion of albumen. The data for effects upon the total shell material are conflicting and of uncertain meaning. The nicotinized eggs obtained from no. A120 (table 1) were the smallest group of the series. Nevertheless the average amount of shell placed upon these eggs was absolutely the largest in amount. For no. A843 (table 2) also the relative proportion of shell material is probably high. For eggs derived from the other two birds (nos. 907, A798, table 2) the amount of shell material is less than normal. The proportion of bases found in this shell material was quite normal.

The data seem to indicate that about one-sixth of the lethal dose of nicotine, given either once or twice daily, neither notably nor wholly consistently affects the amount or gross nature of the shell material secreted.

Pilocarpine. The shells of eggs derived under dosage with pilocarpine were not removed, weighed and analyzed. These eggs were given careful incubation under other birds and the relative thickness or adequacy of their shells estimated by the rate at which such eggs lost weight, i.e., the rate at which the shells permitted the passage of water vapor from the eggs. Previous work (5) has shown that the thinner the shell the more rapid is the rate of loss of weight by the egg, and one purpose of the present study was to further the ultimate discovery of a means of preventing the formation of shells whose rate of loss of water is so high as to be incompatible with complete embryonic development in the egg. In table 4 this rate of loss from treated eggs may be compared with the mean for two corresponding (first or second of clutch) control eggs produced by the same bird immediately before and immediately after the treated eggs.

The table permits the following comparisons concerning eggs whose shells were produced under pilocarpine dosage:

13 (first of clutch) gave a rate of loss of 3.6, against 3.9 for control.
15 (second of clutch) gave a rate of loss of 4.2, against 4.8 for control.
14 (first of clutch) had a mean weight of 8.46, against 8.60 for control.
17 (second of clutch) had a mean weight of 9.14, against 9.16 for control.

Of these 28 eggs which permit comparisons of relative rate of loss of treated and control eggs, 17 treated eggs show a lower rate of loss; 6 treated eggs a higher rate of loss; 5 show equality of rate of loss. Since the treated and control eggs are of equal size the reduced rate of loss from this large proportion of shells produced under pilocarpine is almost certainly not the result of chance. The average reduction of the rate of loss for the 28 treated eggs compared with the controls is exactly 10.0 per cent. For the first eggs of the clutch (which were necessarily subjected to a somewhat shorter period of dosage), the reduction was 7.7 per cent; while for second eggs of the clutch this was 12.5 per cent. The data therefore indicate that the secretion of shell material is measurably increased, or made more adequate, under pilocarpine dosage. It is clear, however, that in the case of certain birds (P891, P853) which were producing abnormally thin shells the pilocarpine dosage wholly failed to induce the secretion of a normal amount of shell material.

The birds grouped at the bottom of table 4 were dosed with pilocarpine throughout the final period of yolk growth in addition to the period of albumen and shell secretion. These eggs therefore give some opportunity to determine whether and how pilocarpine influences total egg size. The thirteen eggs thus treated average 8.81 grams; the thirteen controls, 9.03 grams. On the other hand, nine eggs treated only during the period of albumen or shell secretion average 8.93, their controls 8.78 grams. It is possible therefore that pilocarpine slightly increases the secretion of albumen but reduces the amount of yolk production. The lack of information concerning the yolk-size of these eggs as well as the limited numbers involved makes a decision on the latter point more or less uncertain. The data supply fairly good evidence, however, that the amount of shell material is somewhat increased under pilocarpine and that the amount of albumen is certainly not decreased, but probably slightly increased when the dosage is limited to the period of actual albumen and shell secretion.

The shells of five eggs shown in table 4 were produced under heavy *atropine* dosage and their adequacy was studied by means of their rate of loss as was done in the case of the shells produced under pilocarpine. The numbers involved are here too few to be of much significance. Three of the atropine-treated shells show a lower rate of loss and two of them a higher rate of loss than their respective controls.

Accurate data concerning the time of egg-laying were obtained throughout the present study. This was necessary in order that one might know whether any increase or decrease of the secretions could be accounted for by a longer or shorter period occupied by the passage of the egg down the oviduct. Originally these data were included in all of the tables, but in order to economize space in already overcrowded tabulations these were ultimately omitted. It is well known that at any given season doves deposit their eggs at a markedly definite hour. Our data show a slightly greater irregularity of time of laying under dosage than under control. In case of none of the drugs used, however, was either an acceleration or a delay of the time of laying clearly or consistently obtained. The measured amounts of the various secretions are therefore uninfluenced from this source; it follows moreover that the movements which propel the egg down the oviduct were either practically unaffected by these drugs or, if such motor responses were present, they nevertheless failed to affect the time of the final expulsion of the egg.

DISCUSSION

In any consideration of the activity of the oviduct it is important that one striking peculiarity of this organ be not overlooked. The oviduct passes repeatedly and periodically from a functional to a semi-functional state within the period of one week or slightly more; and during this period the size of the organ may change by no less than 200 to 400 per cent. The functional organ may occasionally attain perhaps even fifty times its non-functional size. This enormous fluctuation in size may have a bearing upon the innervation—or the lack of it—of the gland cells which form a prominent part of the functional organ. The appearance of the actively secreting glandular cells of the fowl has been briefly described by Cushny (9).

In mammals Loeb (10) was able to show that at a definite period after ovulation the internal secretion of the corpus luteum sensitizes the mucosa of the uterus in such a way as to enable it to form the maternal placenta in response to a contact stimulus provided by the ovum. An essentially parallel situation in birds would involve: First, the preparation of the oviduct for its functional state (growth) under the influence of an internal secretion derived from the ovary; second, the active production of albumen and shell (secretion) in such a prepared oviduct in simple response to the contact stimulus supplied by the passing ovum or other foreign body. The meager modification of the amount of albumen and shell secretion obtained by us by means of drugs with presumably pronounced action upon the nerve supply of the oviduct thus affords some evidence that this hypothetical mechanism also best coincides with the facts; and, further, that this mechanism is little if at all directly affected by nervous action.

Several studies with the same drugs used by us have been made upon the motor nerves of the uterus of various mammals but for reasons stated at the beginning of this paper we do not feel in a position to undertake a discussion of the relation of that work to the present study. However, the rather special relation of pilocarpine and atropine to uterine movements in the cat and the rabbit should be noted here. Cushny (11) says of atropine and pilocarpine: "These two drugs seem to affect some structure, which is different from that acted upon by the other drugs examined (nicotine, ergot, adrenalin, quinine), and which does not appear to be involved in the spontaneous contraction and the response to nerve stimulation." Our data indicate a more or less similar action of atropine, cocaine and nicotine, and an opposed action

of pilocarpine, on the oviducal secretions of the bird. In an earlier investigation Riddle and Anderson (12) found, in the same sort of birds used in the present study, that quinine very markedly decreases the secretion of albumen and of egg yolk. They were inclined to interpret this as a result of the nitrogen-conserving action of the drug. It is possible that the small effects obtained with the drugs used in the present study are also due to effects on the general metabolism of the birds; and that, just as atropine and pilocarpine are not supposed to affect lymph formation in mammals, they have also no direct action upon the oviducal secretions of birds. The effect of quinine on shell secretion was not quantitatively studied in the investigation cited above but it is almost certain that no pronounced change was effected.

If atropine retards and pilocarpine accelerates development within relatively undifferentiated cells, as found by Mathews (13) in dividing starfish and sea urchin eggs, it is also possible to consider the rather small observed effects of these drugs upon the oviducal secretions described here as due to a direct action of the drugs upon the secreting cells.

SUMMARY

Atropine administered twice daily and in the dosages used by us decreases the amount of the output of the albumen-secreting glands but the decrease is only about 2 or 3 per cent.

The albumen secreted under atropine is of normal composition with reference to its water content and the relative proportions of alcohol-soluble and alcohol-insoluble constituents.

The amount of shell material secreted under atropine is not measurably affected. The percentage of bases present in the shell ash is unaffected or only slightly reduced.

Cocaine probably decreases the output of the albumen-secreting glands. The amount of shell material was apparently decreased by about 5 per cent under cocaine. The percentage of bases present in the shell ash was not decisively affected.

Nicotine given once and twice daily did not affect, in any constant or definite way, the amount or the gross nature of the shell material secreted. Its effect upon albumen secretion was not adequately tested.

Pilocarpine probably slightly increases the secretion of shell material and, when the time of dosage is properly restricted, of albumen also.

The results indicate that the occasional imperfect functionings of the avian oviduct which result in the production of inadequate egg

shells probably cannot be even temporarily corrected by means of alkaloidal drugs.

The nature of the innervation of the oviducal glands of birds is apparently unknown. Whether the drugs used in this study act upon the autonomic nerves of birds in a way parallel to their action in mammals is very inadequately known. Definite conclusions are not drawn by us as to the relation of effects produced by these drugs to the nature and extent of the innervation of the oviduct.

If the innervation of the oviduct is similar to that of the mammalian uterus, and the alkaloids used by us have an action on the autonomic nerves of birds similar to their action in mammals, these results supply some evidence that the oviducal secretions of birds occur largely independently of the nervous system. The small effects observed are possibly ascribable to the direct action of the drugs on the secreting cells, or to more general action on the metabolism of the animal.

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COMPARATIVE STUDIES OF THE EARLY REACTIONS IN SPINAL CATS PRODUCED BY VARIOUS METHODS

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The unpredictability of shock after spinal transection, in cats, by various methods, stimulated this investigation of the possible relation of the early reactions in spinal cats to the surgical treatment to which such animals are subjected.

Experiments recorded in the literature were performed on various animals for the purpose of observing reflex phenomena over long periods of time The operative procedures were conducted with the animals in surgical anesthesia, which precluded early reflex responses after the spinal cord was transected. Early, frequent and accurate blood pressure determinations were not made.

This investigation was conducted upon cats under very light anesthesia at the time of the spinal transection in order to study reflex phenomena and other reactions, instantly after cutting the cord.

METHOD: *Procedures common to all series.* The animals were completely anesthetized with ether from a cone. The blood pressure was taken at intervals throughout the experiments from the left femoral artery, instead of either carotid, to prevent interference of the cannula with subsequent operation in the neck.

The ether was discontinued, and after anesthesia became very light, the cord was cut.

Transection was effected as quickly as possible to preclude hemorrhage.

Reactions to the following stimuli were observed, immediately after the spinal cord was severed, and from time to time throughout the experiment: *a*, sudden pinching of the toes of the hind feet, *b*, pinching of the skin of the hind legs and abdomen, *c*, constant and intermittent pressure against the plantar surface of the hind feet, *d*, scratch irritation of the girdle, *e*, suspension of the animal by the skin of its back,

f, pinching of different portions of the tail as a whole, and of various skin areas of the same, *g*, pinching of the testes singly and together, *h*, striking the various tendons from the pelvis to the toes, inclusive.

The incidence, duration and type of reflex response were carefully observed after the cord was severed.

The length of time the animals lived, after the transection, was also observed.

The spinal canal was carefully opened post mortem, to determine the success and location of the transection.

Further procedures differed in the three series.

Series I. The right vertebral and carotid arteries were ligated, and followed by ligation of the left vertebral and carotid arteries. The cats were tracheotomized and given artificial respiration. Transection of the spinal cord was done in the region of the first cervical segment.

Series II. The cats were given artificial respiration, and the cord was severed in the region of the first cervical segment.

Series III. Without either ligation of the vertebral and carotid arteries or artificial respiration, the spinal cord was divided between the second and third dorsal segments.

RESULTS: *Series I. Blood pressure variations.* The initial blood pressure in this series of twelve animals averaged 153 mm. Hg. (table 1).

The blood pressure in nine of the animals was depressed, on an average, 31 mm. Hg. (from 2 to 66 mm. Hg.) following ligation of the right vertebral and carotid arteries. This represents a percentage fall from the original average blood pressure of 20.9 per cent. Blood pressure in one animal was not determined at this period. The remaining two animals, however, showed increases of 17 to 28 mm. Hg., respectively.

When in addition to ligation of the right vertebral and carotid arteries, the left vertebral and carotid arteries were ligated, the blood pressure rose on the average of 17 mm. above the initial pressure. On the whole, therefore, ligation of the right vertebral and carotid arteries lowered the blood pressure below the original level, and subsequent ligation of the left vertebral and carotid arteries raised the pressure, not only to the initial pressure level, but above it slightly.

Immediately after completion of these ligations artificial respiration, by tracheal insufflation, was instituted. This procedure while slightly reducing the blood pressure did not depress it below the initial level.

Transection of the spinal cord in the region of the first cervical segment followed. After a lapse of 2 minutes, on the average, the blood

pressure became constant on a new but lower level. The average level following this procedure was 67.5 mm. Hg in contradistinction to a pressure of 153 mm. Hg initially, and 154.9 mm. Hg just before the cord was cut. The blood pressure, therefore, was reduced some 85 mm. Hg or approximately 55 per cent as an immediate result of the transection.

Temperature. The fall of temperature of the body surface in the animals living as long as 15 or 20 minutes after severing the cord was

TABLE 1

Series I. Blood pressure variations

EXPERIMENT	INITIAL BLOOD PRESSURE	BLOOD PRESSURE AFTER TRANSECTION	ABSOLUTE FALL FROM INITIAL BLOOD PRESSURE	PER CENT FALL FROM INITIAL BLOOD PRESSURE	TIME LIVED AFTER TRANSECTION	PER CENT FALL OF BLOOD PRESSURE IN CATS NOT GIVING REFLEXES IMMEDIATELY AFTER TRANSECTION	PER CENT FALL OF BLOOD PRESSURE IN CATS GIVING REFLEXES IMMEDIATELY AFTER TRANSECTION
no.	*mm. Hg*	*mm. Hg*	*mm. Hg*	*per cent*	*minutes*	*per cent*	*per cent*
1	184	56	128	69.5	3	69.5	
2	144	84	60	41.6	2	41.6	
3	198	86	112	56.5	3	56.5	
4	142	40	102	71.8	7		71.8
5	144	76	68	47.2	134*		47.2
6	114	64	50	43.7	58		43.7
7	156	130	26	16.6	54*		16.6
8	154	35	119	77.2	120	77.2	
9	136	36	100	73.5	14		73.5
10	178	60	118	66.2	57*	66.2	
11	130	64	66	50.7	44		50.7
12	156	80	76	48.7	5		48.7
Average...	153	67.5	85.4	56.1	28.4†	62.2	50.3

* Experiment interrupted before animal died.

† Average life of those that died.

sufficient to detect, unquestionably, by touching the surface of the animal.

Reflexes. Instantly after the spinal transection, five of the animals showed no muscle tonus or reflex response of any kind (table 2). The remaining seven of the twelve animals responded with at least one type of reflex.

Relation of blood pressure to the appearance of reflexes immediately after transection of the spinal cord. The relative individual depressions

from the initial pressure in the five animals giving no reflex response ranged from 41.6 per cent to 77.2 per cent (table 1), and those in the seven animals giving reflex response ranged from 16.6 per cent to 73.5 per cent.

Fate of the animals. The duration of life of the animals of this series was, for the most part, short. Of the twelve animals, nine died on an average of 28.4 minutes (varied from 2 to 120 minutes) after the cord was cut. The remaining three were killed at periods of 134, 57 and 54 minutes after the spinal transection.

TABLE 2

Series I. Reflexes after cutting cord

EXPERIMENT	RESPONSE IMMEDIATELY AFTER TRANSECTION	TIME OF APPEARANCE OF REFLEXES AFTER TRANSECTION	DURATION OF REFLEXES	DURATION OF LIFE AFTER TRANSECTION	TRANSECTION
no.		*minutes*	*minutes*	*minutes*	
1	None		0	3	Complete
2	None		0	2	Complete
3	None	2	1	3	Complete
4	Reflexes	Immediately	1	7	Complete
5	Reflexes	Immediately	58	134†	Complete
6	Reflexes	Immediately	5	58	Incomplete
7	Reflexes	Immediately	29*	54†	Incomplete
8	None	10	45	120	Complete
9	Reflexes	Immediately	11	14	Complete
10	None	7	48*	57†	Complete
11	Reflexes	Immediately	42	44	Complete
12	Reflexes	Immediately	3	5	Incomplete

* Experiment interrupted at this time, reflexes still active.

† Experiment interrupted before animal died.

Post-mortem examination. Three animals in this series showed almost but not quite complete transection of the cord.

Series II: Blood pressure variations. In this series of six cats the average initial blood pressure was 154.6 mm. Hg. (table 3).

Institution of artificial respiration, by tracheal insufflation, was followed by a decrease of blood pressure to 127.2 mm. Hg.

The spinal cord was then transected in the region of the first cervical segment. Within 2 minutes the average absolute blood pressure was constant at 89.6 mm. Hg. The pressure, therefore, was now 65 mm. below the initial level, which represents a relative depression of 42 per cent.

Temperature. There was a decrease of body surface temperature 15 or 20 minutes after cutting the cord sufficient to detect by palpation.

Reflexes. Each of the six animals gave flexion and extension instantly after transection of the cord (table 4).

TABLE 3

Series II. Blood pressure variations

EXPERIMENT	INITIAL BLOOD PRESSURE	BLOOD PRESSURE AFTER TRANSECTION	ABSOLUTE FALL FROM INITIAL BLOOD PRESSURE	PER CENT FALL FROM INITIAL BLOOD PRESSURE	TIME LIVED AFTER TRANSECTION
no.	*mm.* Hg	*mm.* Hg	*mm.* Hg	*per cent*	*minutes*
1	146	120	26	17.8	11
2	204	90	114	55.8	39
3	190	92	98	51.3	42
4	144	60	84	58.3	20
5	148	142	6	4.0	10
6	96	34	62	64.5	100*
Average.....	154.6	89.6	65	41.9	24.4†

* Experiment interrupted before death occurred.

† Average life of those that died.

TABLE 4

Series II. Reflexes after cutting cord

EXPERIMENT	RESPONSE IMMEDIATELY AFTER TRANSECTION	DURATION OF REFLEXES	DURATION OF LIFE AFTER TRANSECTION	TRANSECTION
no.		*minutes*	*minutes*	
1	Reflexes	5	11	Complete
2	Reflexes	37*	39	Complete
3	Reflexes	38	42	Complete
4	Reflexes	19*	20	Complete
5	Reflexes	10	10	Incomplete
6	Reflexes	93–99*	100†	Complete

* Reflexes were present at this time but had been lost for a part of the period of observation.

† Experiment interrupted while reflexes were absent.

Relation of blood pressure to the appearance of reflexes immediately after transection of the spinal cord. The individual absolute depressions from the initial blood pressure ranged from 6 to 114 mm. Hg, and the individual relative falls ranged from 4 per cent to 64.5 per cent. All of the animals gave reflex response.

Fate of the animals. The duration of life was short in this series, five of the animals dying on an average of 24.2 (varied from 10 to 42 minutes) minutes after the spinal transection. One animal was killed at the expiration of 100 minutes.

Post-mortem examination. One animal of the series showed not quite complete transection of the cord.

Series III: Blood pressure variations. The initial blood pressure in this series of eight animals averaged 173.7 mm. Hg (table 5).

Without further operative procedures, the spinal cord was transected between the second and third dorsal segments.

TABLE 5

Series III. Blood pressure variations

EXPERIMENT	INITIAL BLOOD PRESSURE	BLOOD PRESSURE AFTER TRANSECTION	ABSOLUTE FALL FROM INITIAL BLOOD PRESSURE	PER CENT FALL FROM INITIAL BLOOD PRESSURE	TIME LIVED AFTER TRANSECTION
no.	*mm. Hg*	*mm. Hg*	*mm. Hg*	*per cent*	*minutes*
1	162	156	6	3.7	48 hrs.*
2	178	150	28	15.7	120*
3	168	75	93	55.3	125*
4	190	152	38	20.0	82*
5	154	92	62	40.2	30*
6	164	106	58	35.3	112*
7	194	76	118	60.8	39*
8	180	114	66	36.6	44*
Average.....	173.7	115.1	58.6	33.7	

* Experiment interrupted at this time.

After an average interval of 2 minutes the blood pressure was stabilized at 115.1 mm. Hg. The absolute decrease from the initial level averaged 58 mm. Hg, and the relative depressions averaged 33.7 per cent.

Temperature. Although accurate determinations of the body surface temperature were not made throughout this investigation, the animals of this series did not manifest sufficient temperature variations to detect by feeling the surface of the body.

Reflexes. Each member of this series gave two or more types of reflex response instantly after severing the cord (table 6).

Relation of blood pressure to the appearance of reflexes immediately after transection of the spinal cord. The individual absolute depressions from the initial blood pressure ranged from 6 to 118 mm. Hg, and the

individual relative depressions from the same level ranged from 3.7 per cent to 60.8 per cent. All of the animals, however, gave reflex response under these various conditions of blood pressure.

Fate of the animals. Not one animal died during the periods of observation, which varied from 34 minutes to 48 hours. The usual period of observation, however, approximated 2 hours.

Post-mortem examination. The spinal cords of three animals of this series were not quite completely severed.

Note: The reactions in those animals undergoing almost but not quite complete transection were so comparable to those of the cats after complete severance of the cord that no attempt has been made to separate the two classes.

TABLE 6

Series III. Reflexes after cutting cord

EXPERIMENT	RESPONSE IMMEDIATELY AFTER TRANSECTION	DURATION OF REFLEXES	TRANSECTION
no.		*minutes*	
1	Reflexes	48 hrs.*	Complete
2	Reflexes	120*	Complete
3	Reflexes	125*	Complete
4	Reflexes	82*	Complete
5	Reflexes	30*	Incomplete
6	Reflexes	112*†	Incomplete
7	Reflexes	39*	Incomplete
8	Reflexes	44*	Complete

* Experiment interrupted but reflexes were still active.

† Reflexes were present at this time but had been lost for a part of the period of observation.

SUMMARY AND CONCLUSIONS

1. Ligation of the vertebral and carotid arteries, before severing the spinal cord, does not reduce the amount of blood loss.

2. The blood lost after spinal transection, with or without previous ligation of the vertebral and carotid arteries, is negligible.

3. The blood pressure begins to fall immediately after severing the cord and becomes constant at its new level within 2 minutes.

4. Spinal transection in the region of the first cervical segment, under very light ether anesthesia and artificial respiration, but without ligation of the vertebral and carotid arteries, produces appreciably less depression of blood pressure than spinal transection under the same conditions, with ligation of the vertebral and carotid arteries.

5. Transection of the spinal cord between the second and third dorsal segments, under very light ether anesthesia and without ligation of the vertebral and carotid arteries or artificial respiration, produces distinctly less fall of blood pressure than when the spinal transection is made in the region of the first cervical segment, under very light ether anesthesia and artificial respiration, with or without ligation of the vertebral and carotid arteries.

6. When the cord is transected between the second and third dorsal segments the length of life of the animals indicates no impairment of respiratory function. This is contrary to the findings of Gotch and Horsley (1).

7. There is no relationship, within limits, between spinal shock and blood pressure.

8. The incidence of spinal shock, in cats, is unpredictable, when the spinal transection is made in the region of the first cervical segment, under very light ether anesthesia and artificial respiration, subsequent to ligation of the vertebral and carotid arteries.

9. When spinal transection, in cats, is made in the region of the first cervical segment, under very light ether anesthesia and artificial respiration, without ligation of the vertebral and carotid arteries, spinal shock does not occur.

10. Spinal shock does not occur, in cats, when the spinal cord is severed between the second and third dorsal segments, under very light ether anesthesia and without ligation of the vertebral and carotid arteries or artificial respiration.

11. From the standpoint of the absence of spinal shock after spinal transection there can be no doubt that the best preparations are those made without previous ligation of the vertebral and carotid arteries. While the evidence strongly indicates a relationship between spinal shock and the surgical trauma preceding spinal transection, it is felt that the data are inadequate to permit of such a definite conclusion at this time.

12. In general, the length of life after spinal transection is much greater when the cord is severed between the second and third dorsal segments, when no ligations to control hemorrhage are made, and no artificial respiration is given.

13. Body temperature falls appreciably in cats undergoing spinal transection in the region of the first cervical segment.

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STUDIES ON THE VISCERAL SENSORY NERVOUS SYSTEM

X. The Vagus Control of the Esophagus

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This reinvestigation of the motor control of the esophagus by the vagus nerves was prompted by the unexpected type of control by the vagi of the lung motor mechanism in the amphibia (frogs and salamanders). It was found that the primitive amphibian lung possesses independent or peripheral automatism like the heart and the gut and, as in the case of the heart, the local lung automatism is normally prevented from full development by tonic inhibition via the vagi nerves (6), (20). The lungs are diverticula of the esophagus. The question naturally arises whether in the adult stage of any species of mammals the esophagus, or any region of it, retains the type of local automatism and extrinsic inhibitory control shown by the amphibian lung. This question appears to us of peculiar importance in relation to the phenomena of cardiospasm and spasm of the esophagus in man.

It was pointed out in one of our earlier papers that the type of motor control (exclusively inhibitory) found in the most primitive lung available for study (lung of necturus) is probably an original state rather than a result of differentiation, because in the higher amphibia (frogs), with their more developed lungs, motor nerve fibers are present as part of the extrinsic nerve mechanism, while in the reptilia the local lung automatism is but poorly developed, the extrinsic innervation is of the motor type, the inhibitory type being very subordinate, if not completely lacking (7).

It is also evident that the vertebrate esophagus has, at least in some species, undergone a greater degree of differentiation than the lung motor mechanism. In some species the musculature of the entire esophagus has changed to the striated type, and parallel with this change there is absence of Auerbach's plexus and other local ganglia. In other species this transformation of the primitive esophagus has so

far involved only the upper two thirds of this organ. Obviously these changes in structure are paralleled by changes in type of automatism and motor control, but the latter changes may come about in the absence of change in gross structure, as shown by the amphibian and reptilian lung. When the smooth musculature and local nervous system is retained, as is the case with the cardiac and pyloric sphincters, the degree of deviation from the primitive control is a matter for experimental determination. If the primitive inhibitory control of the cardia predominates in a species, cardiospasm could be induced, not by "vagotonia," as is at present held by many clinicians, but by vagus "hypotonia," that is, failure of the tonic vagus inhibition of the cardia. We have not gone extensively into the clinical literature on cardiospasm, but it is well known that many patients with this malady exhibit other symptoms of impaired inhibitions and Strock (32) quotes Kraus as having shown actual atrophy of the vagi in a case of cardiospasm.

LITERATURE

In 1861 Ravitsch (27) reported that section of both vagi in the frog tends to paralysis of the stomach. Ten years later Goltz (12) showed that in frogs destruction of the medulla by pithing it or killing it chemically with huge doses of curare or as the result of inhalation of chloroform caused a markedly hypertonic condition of the esophagus and stomach together with a great increase in the motility of these structures. This hypertonus of the esophagus and stomach with increased motility occurred also after double vagotomy. Once produced the condition was permanent, relaxation of these organs occurring only when the tissues were dead (acute experiments). Goltz therefore assumed that the vagi carried inhibitory fibers to the esophagus and stomach which were in tonic activity. To his great surprise stimulation of the peripheral vagus trunk did not cause the anticipated inhibition of motility but rather augmented the motility of the esophagus and stomach. Goltz furthermore showed that this hypertonus and increased motility of the stomach could be induced by chemical, mechanical, thermal and electrical stimulation of different parts of the skin and small intestines. Goltz, however, did not consider these effects as types of reflex activity but presumed that the intense stimuli employed paralyzed more or less completely the medullary center from which under normal conditions the tonic inhibitory impulses for the esophagus and stomach arose ("Herabsetzung der Lebensenergie"). It seems to be essentially a reflex inhibition of the tonic activity of the medullary center

quite similar to the reflex inhibition of the inhibitory lung motor center of the frog and other amphibia previously described by us (6), (20). Since it was shown that the vagus on direct stimulation caused an increase in the hypertonus and motility which followed its division, it is possible that the hypertonus and increased motility of the esophagus and stomach which arose on sensory stimulation of cutaneous and visceral nerves was in part due to the reflex stimulation of these motor fibers of the vagus together with central inhibition of the activity of the sympathetic fibers carried to the stomach by the splanchnics.

The original observations of Goltz were confirmed and extended by Contejean (9) in 1892 and by Steinach (30) in 1898. Relying on direct observations, Steinach found that on destruction of the medulla or section of the vagi nerves in the frog (R. esculenta), the esophagus passes at once into permanent hypertonus, the stomach and the upper end of the small intestine show increased spontaneous motility and increased excitability to direct stimulation as well as to stimulation of the upper spinal nerves. Steinach varied the experiments by local etherization of the medulla. In this way Steinach found that the hypertonicity and hypermotility of the upper end of the alimentary tract can be induced two or three times in the same animal and the normal conditions restored by removing the ether from the medulla by the aid of irrigation with physiological salt solution. Steinach states that after the third or fourth period of etherization the medulla cannot be restored to normal. Steinach was less successful in causing inhibition of the gastric and esophageal tonus by electrical stimulation of the medulla, but as he relied on direct observation, slight tonus changes following stimulation of the medulla could not be detected. Steinach concluded that in the frog the vagi contain inhibitory fibers for the esophagus, the stomach, and the upper end of the small intestines, and that these fibers are in tonic activity, like the vagus inhibitory nerves to the heart.

Bottazzi (3) reported motor effects but no distinct inhibition of the esophagus of the toad on stimulation of the vagi and the sympathetic nerves. On stimulation of the pure vagi the contraction of the longitudinal musculature predominated.

Stiles (31) showed that isolated segments of the frog's esophagus exhibit a greater degree of spontaneous automatism than similar segments from other regions of the alimentary tract. The reactions of these esophagus segments (circular fibers) to solutions of electrolytes parallel those of the heart.

Waddell (33) found that pituitrin has a primary depressant action on the frog's esophagus, the depression appearing both in the longitudinal and the circular musculature. It is most marked in the cardiac end of the esophagus.

In 1911 Hopf (13) reported extensive experiments on the effect of vagi stimulation on the stomach and esophagus of the frog, concluding that the vagi contain both inhibitory and motor fibers to the esophagus and stomach, the motor fibers predominating. Hopf stated also that if the esophagus and cardia are put on too great tension the inhibitory action of the vagi on these structures cannot be demonstrated. Unfortunately the experimental procedure followed by Hopf is open to objection, and the numerous tracings published are far from convincing in support of his conclusions.

Patterson (26) found that section of both vagi (vago-sympathetic) in the bull frog rendered the stomach hypertonic for a period of 10 to 15 days. The condition of the esophagus and cardia was not noted.

There appears to be nothing in the literature on the effects of vagus section on the esophagus and cardia in the reptilia. Bercovitz and Rogers (1) have recently shown that section of the vagi in the turtle may increase the gastric tonus, at least temporarily, and stimulation of the peripheral vagus with weak induction shocks repeated at slow rate inhibit both gastric tonus and gastric peristalsis, while stronger stimulation of the vagi induces gastric contractions. This indicates that in the turtle the vagi contain both motor and inhibitory fibers to the stomach.

Doyon (10) reported that appropriate stimulation of the peripheral vagus in the pigeon inhibits the motility of the gizzard, and that this inhibitory action is abolished by nicotin.

Observations on mammals are more numerous. Bernard (2) and Schiff (28) reported a temporary (10 to 72 hours) spasm of the esophagus and cardia following section of the vagi in dogs. Chauveau (8) noted a similar spasm of the esophagus in three horses following double vagotomy. In all horses double vagotomy was followed by paralysis of the esophagus.

Kronecker and Meltzer (17) reported reflex dilatation of the cardia, but ascribed this to central inhibition of the vagus motor tonus to the cardia. Later (1906) Meltzer and Auer (23) showed that the vagus contains inhibitory fibers to the cardia of the rabbit and that these can be excited reflexly by stimulation of the central end of the remaining vagus.

Openchowski (24), (25) described in the rabbit, dog and cat a branch of the vagus on the lower end of the esophagus, whose stimulation caused dilatation of the cardia. This nerve was accordingly called "nervus dilator cardia." The dilatation of the cardia was also obtained by stimulation of the peripheral end of the vagus with weak induction shocks of slow rhythm. With stronger shocks at a rapid rate contraction of the cardia was induced.

This investigator also describes groups of ganglion cells, in addition to the plexus of Auerbach, in the region of the cardia. These nerve cell groups are similar in structure to those of the heart ganglia. He concludes further, on the basis of experiments on rabbits, dogs and cats, that there is a constrictor center for the cardia in the posterior corpora quadrigemina, while the primary dilator center for the cardia is located in the region of the union of the nucleus caudatus and nucleus lenticularis. Most of the constrictor fibers for the cardia pass out in the vagi, principally the left, but a few reach the cardia via the cervical nerves and the cervical sympathetic. Some of the dilator fibers for the cardia also pass out through the cervical nerve roots and reach the cardia via the aortic plexus.

Openchowski states that, with the vagi intact, reflex dilatation of the cardia may be induced by stimulation of the kidneys, the uterus, the urinary bladder, the intestines or the sciatic nerve. Dilatation of the cardia is sometimes induced by stimulation of the cerebral cortex in the region of the crucial sulcus.

Langley (18), working with curarized and atropinized rabbits, reported that stimulation of the peripheral vagus caused dilatation of the cardia. When no atropin was administered the vagus stimulation caused increased tonus of the cardia. Langley therefore concluded that the vagus nerves carry both motor and inhibitory fibers to the cardia, the motor fibers being more readily paralyzed by atropin. Later Langley (19) reported that the adrenalin inhibits the cardiac sphincter in the rabbit.

Krehl (16) reported that section of both vagi in the dog left the esophagus and the cardia atonic or patulous. The state of the cardia seems to have been determined by the absence of resistance to the passage of a stomach tube into the esophagus through a gastrostomy. These findings of Krehl on the dog are accepted, without additional proofs, by Katschowsky (14), working in Pawlow's laboratory.

On the basis of the examination of four dogs, Sinnhuber (29) reached the conclusion that section of the vagi above but close to the diaphragm

renders the cardia atonic, while section of the vagi high up in the neck leads to a temporary hypertonicity of the cardia. If these observations should prove to be correct, it would seem that in the dog the inhibitory fibers to the cardia leave the vagi at some distance above the diaphragm and pass down to the cardia in the wall of the esophagus.

Espezel (11) declared (in 1901) that inhibitory nerve fibers to the esophagus had not yet been satisfactorily demonstrated, at least for the rabbit and the dog.

Strock (32), working on dogs, reported that section of both vagi causes dilatation of the esophagus, and a slight increase in the tonus of the cardia. He quotes V. Mickulicz to the effect that section of the vagi doubles the tonus of the esophagus as measured by the resistance to the forcing of liquids through into the stomach. Nevertheless Strock does not believe that this hypertonus of the cardia in dogs is analogous to clinical cardiospasm.

Kelling 15) states that deep anesthesia increases the resistance of the cardia to pressure exerted from the stomach side. If we assume that the anesthetic paralyzes the medullary vagus center before paralysis of the local motor mechanism of the cardia, one may conclude that the cardiospasm induced by deep anesthesia is caused by elimination of the vagi inhibitory impulses to the cardia.

Cannon (4) reported (1907) that section of both vagi in the cat leads to an increased tonus of the cardia lasting for several days (and in some cases indefinitely), parallel with decreased tonus and peristalsis of the esophagus. This conclusion was based on the observation that the peristalsis of the lower esophagus frequently failed to force the food through the cardia, and the increased resistance at the cardia to the passage of the stomach tube.

The resistance of the cardia to the passage of food may not indicate any increased tonus of the cardia, but merely the failure or absence of the normal inhibitory reflex. We do not believe that under normal conditions the cardia is forced mechanically by the strength of the esophagus peristalsis. It seems more probable that the passage of food through the cardia in normal swallowing is associated with reflex inhibition of the tonus of the cardia. Furthermore, the degree of resistance offered to the passage of the stomach tube is subject to many factors, so that actual quantitative differences, even if established, are capable of more than one interpretation.

EXPERIMENTAL METHODS

Turtles. In practically every case the animals were decerebrated as a preliminary step, to avoid the use of anesthetics in subsequent operations. In a few experiments the spinal cord was sectioned below the medulla, leaving the brain intact. Since all subsequent dissection is made peripheral to the spinal transection, no pain impulses can reach the brain.

The animal is secured, ventral side down, on the turtle stand, in a manner described in a previous communication (7). The entire spinal cord is pithed, the spinal column of the neck and the large retractor neck muscles excised. We usually made a window through the carapace on the left side, exposing the left lung and the stomach. The left lung was prepared for the administration of artificial respiration.

Three methods of introducing and adjusting the delicate balloons in the esophagus and the stomach were tried out. Through a small slit in the stomach 2 cm. from the cardia a flexible seeker was passed through the esophagus; the gastric and esophageal balloons were attached to this seeker and pulled back into their respective positions, the flexible rubber tubes connecting the balloons with the respective water manometers. This method has the disadvantages of the trauma to the stomach wall, the mechanical action on the rubber tubes of the swallowing or respiratory movements of the jaws, and possible reflex influence on the esophagus and stomach (via the medulla) from the irritation of the tubes in the mouth and pharynx. To avoid these latter factors we made a small opening in the wall of the esophagus just below the pharynx and passed the tubes from the manometers through this slit, thus leaving the mouth free from mechanical stimulation.

In a few experiments the stomach was left intact, the balloons being pulled through the esophagus and stomach into their proper positions through a slit in the duodenum near the pylorus. We also pushed the balloons into position by means of a seeker operated through the esophagus. This leaves the stomach intact. When records were taken from the esophagus only, the balloon was either pushed into position by means of a seeker operated through the mouth or pulled into place from an opening into the stomach.

The tubes connecting the balloons must be anchored at the head end to prevent them from being pushed from the esophagus into the stomach, and from the stomach into the intestine by the esophageal and gastric peristalses. Since by our methods of preparation both esophagus and

stomach are exposed from the dorsal side, moderate inflation of the balloons will disclose their exact location by direct inspection. Furthermore, the exact location of the respective balloons was verified by opening the esophagus and stomach at the end of each experiment.

A few observations were made on the action of the vagus nerves on the esophagus, after removal of the esophagus from the body. The excised esophagus was kept moist with Ringer's solution but no attempt was made to perfuse the organ.

It is scarcely necessary to state that there is considerable hemorrhage in the turtles as a result of the extensive dissection required to remove all external influences on the upper end of the gut. There was accordingly considerable impairment of the circulation. Impaired circulation in the esophagus and stomach may not only induce abnormal motor activities, but will be a source of error in a study of the action of drugs, when these are injected intravenously. To control these sources of error the loss of blood was compensated for by intravenous injection of Ringer's solution. One can judge the state of the circulation in our preparations fairly accurately by direct inspection, and in all cases of doubt methylene blue was injected intravenously at the end of the experiments, in order to check up on the efficiency of the circulation in the organs concerned.

The pithing of the brain (medulla) was usually made from the spinal cord end. The vagi sections were at a distance of 0.5 to 1.0 cm. from the angle of the jaw. The pulmonary vagi were usually sectioned so that the lung contractions caused by stimulation of the vagi may not influence the tension in the stomach and esophagus.

The esophageal and gastric balloons were made out of rubber condoms, the length of the balloons varying with the size of the animal. The initial pressure in the balloons was usualy fixed at 1 to 2 cm. of water, the graphic registration being in every case made by means water manometers (diameter, 8-10 mm.).

Frogs. The animals were decerebrated, the spinal cord sectioned in the second cervical segment and pithed posteriorly. The animal is thus immobilized, except for the jaws and the pharynx, and there is no call for anesthesia.

In further preparation the animal was fixed, dorsal side down, and an incision made in the median line from the symphysis pubis to the symphysis of the lower jaws, the body wall pinned to the side, and the esophagus and stomach exposed by pushing the left lung and the liver toward the right side. In some cases the left lung was excised after ligation at the base.

Vigorous animals will usually carry on fairly efficient respiratory movements even after this extensive dissection. But we usually placed a cannula in the tip of the right lung for the purpose of artificial respiration when required.

The necessary dissection causes, of course, hemorrhage in varying amounts, and consequent impairment of the circulation. This was counteracted, so far as possible, by introducing Ringer's solution through the median abdominal vein.

With frogs fixed dorsal side down pithing of the medulla from the spinal cord is not feasible. Hence in the experiments involving pithing of the medulla we usually (after the decerebration) cut off the upper mandible at the level of the anterior end of the brain cavity, so that the pithing needle could be readily introduced and pushed down to the medulla with minimum mechanical disturbance of the graphic registrations. In a few experiments we destroyed the medulla by crushing the skull by strong artery forceps, but the procedure cannot be carried out without some direct mechanical stimulation of the pharynx. In several of the experiments the medulla was destroyed by injecting into it through the anterior end of the cranial cavity one drop of chloroform. This method has the advantage of the sudden destruction of medulla without the movements of the head and neck which always occur during pithing it. It furthermore does not bring about a change in the level of graphic registration as does placing the jaws of the hemostat prior to crushing of the medulla.

In animals thus prepared, the effects on the esophagus by pithing the medulla, sectioning the cervical sympathetic and the vagus nerves, and stimulation of the vago-sympathetic nerves, were studied both by direct inspection and by graphic methods. The latter consisted in placing a hook, connected by a recording lever, under the cardiac end of the esophagus, the stomach being left intact, the pharyngeal end of the esophagus serving as the fixed point. We also sectioned the stomach just below the cardia, fixed a hook in the small stomach segment and suspended the esophagus by the weight of the recording lever, the pharyngeal end again serving as the fixed point. But finding that section of the stomach near the cardia induced motor disturbances in the esophagus, we endeavored to eliminate this by suspending both stomach and esophagus by a hook in the duodenum close to the pylorus. Such records are composites of gastric and esophageal motility. These methods of suspension interfere more or less seriously with the circulation in the esophagus and stomach. But any method of graphic regis-

tration of esophageal motility in our preparations introduces serious errors from the movements of the pharynx. These cannot be eliminated, if the medulla is to be kept in functional activity. The data secured by these graphic methods must therefore be checked against those from direct observation. The frog's esophagus is too short to permit recording tonus and motility by the balloon method.

An aspiration bottle containing Ringer's solution was so arranged as to deliver its contents drop by drop to the surface of the stomach and esophagus. In this way we prevented these structures from drying. In those experiments where the action of drugs was studied the irrigation of the esophagus with Ringer's solution was stopped while irrigating the preparation drop by drop with the drug contained in a hypodermic syringe.

The graphic method employed by us introduces an additional disturbing factor, namely, the local or direct stimulating action of tension on the stomach and esophagus, and the failure to record satisfactorily the tonus and contractions of the circular musculature. In order to eliminate the direct stimulating action of tension on the stomach and esophagus, we allowed these structures to remain in their normal position in the abdominal cavity gently hooking back only such structures which hid them from complete view. We next mounted above the preparation a camera. This latter was essentially a Woelfel artificial eye used ordinarily for classroom instruction in physiological optics. We replaced the stationary ground glass "retina" with larger pieces of ground glass which could be readily moved laterally. The ground surface of the glass was uppermost. The light from two ordinary head lights with reflectors was directed toward the preparation. The room was now darkened and the observer, with head and shoulders under a black cloth, looking at the ground glass surface could, by a little focusing, obtain a distinct and slightly enlarged image of the stomach and esophagus. The outlines of these organs were traced on the ground glass with a soft pencil. As soon as this simple sketch was complete the glass was shifted laterally and another sketch was made. After several sketches were made in this manner the vagi were ligated in the neck, and the stomach and esophagus were sketched many times in rapid succession. In this manner we obtained tracings of the outlines of the esophagus and stomach similar to those obtained by Cannon when studying esophageal, gastric and intestinal movements in mammals over x-rays and under a fluoroscopic screen. It took less than 5 seconds to complete a single sketch. These sketches were subsequently traced

on paper by placing tissue paper over the ground glass plate and illuminating the latter from below with desk lamp.

In the observations on drug actions, the drugs (in Ringer's solution) were applied to the surface of the esophagus; injected intravenously; or injected in large doses hypodermically at different times before the preparation of the animals.

It is of some importance that all solutions used for intravenous injections or superficial irrigation have the same temperature as the frog (approximately room temperature); for heat accelerates and cold depresses the peripheral automatic rhythm seen after ligation of the vagus, destruction of the medulla, or stimulation of the peripheral end of the vagus nerve.

RESULTS ON TURTLES

Effects of pithing the medulla or sectioning of the vagi nerves. If the circulation is maintained in good or fair condition the animal, with only medulla, midbrain and vagi intact, usually executes periodic respiratory (swallowing) movements for hours. These swallowing movements of the jaws are accompanied by contraction of the striated muscles of the pharynx. The contractions of these muscles together with the air pressure in pharynx and upper end of the esophagus cause rapid increase in the pressure in the esophageal balloon, shown in figure 1, *R*. But the respiratory act or periods of swallowing appear to be accompanied by actual decrease in the tonus of the esophagus, and the tonus of the esophagus increases gradually between the swallowing periods (fig.1). In the case of preparations that exhibit no spontaneous respiratory or swallowing movements there is usually no distinct rhythm of the tonus of the esophagus so long as the vagi and the medulla are intact.

The pithing of the medulla or sectioning of the vagi close to their exit from the skull induces a very brief inhibition of the tonus of the esophagus, followed by a permenent hypertonus. Typical records showing these reactions are reproduced in figures 1, *B*, and 2. In some preparations the hypertonus of the esophagus following isolation from the medulla exhibited a slow rhythm (fig. 2, *B*). We were unable to determine whether or not this tonus rhythm was peristaltic contractions of the esophagus. But the hypertonus was rarely equal in degree throughout the entire length of the organ. In other words, isolation of the esophagus from the central nervous system not only induces a general hypertonus, but permits also development of local spasms of the esophagus, lasting in many cases throughout the experiment (6 to 36 hours).

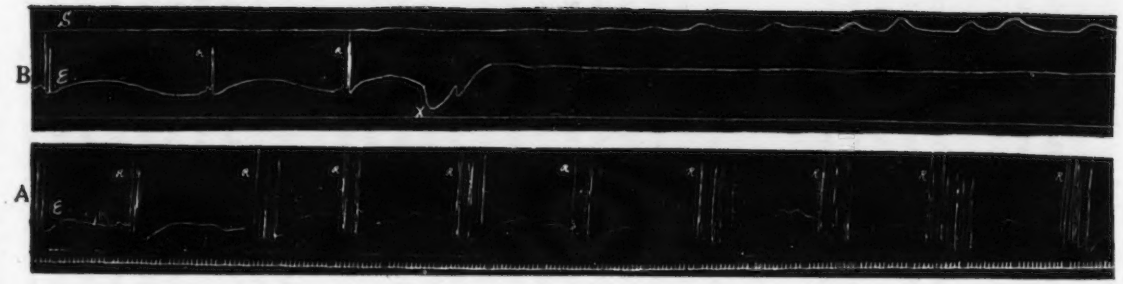

Fig. 1. Turtle decerebrated. Records from the stomach, *s*, and esophagus *e; x*, pithing of the medulla; *r*, swallowing movements (respiration). *A*, showing tonus rhythm of the esophagus inhibited by the swallowing acts; *B*, hypertonus of the esophagus and initiation of gastric contractions by pithing of the medulla.

Fig. 2. Turtle decerebrated. Record of tonus of the esophagus. *A*, *x*, section of both vagi near the head. *B*, *x*, pithing of the medulla. Showing permanent hypertonus and tonus rhythm, *B*, of the esophagus isolated from the brain.

In preparations also provided with a balloon in the stomach we sometimes observed that destruction of the medulla or section of the vagi induced a rhythm in the stomach (fig. 1, *B*). This confirms the recent observations of Bercovitz and Rogers. It should be noted, however, that pithing of the medulla does not invariably augment the tonus in the active or induce a rhythm in the quiescent stomach, but the hypertonus of the esophagus is always induced by this operation, provided the preparation or the animal is not moribund.

The hypertonus of the esophagus shown in figures 1 and 2 seems to involve mainly the circular musculature. There is no distinct shortening of the esophagus. The esophageal hypertonus seemed to extend to the cardia, but we cannot prove this graphically, as it is not possible to confine a balloon in the cardia without it being influenced by the tonus and contractions both of the gastric and the esophageal musculature.

We desire to point out the similarity of the hypertonus of isolated esophagus of the turtle to the hypertonus and tetanus of the amphibian lung following the same operation, that is, sectioning of the vagi or pithing the medulla

Effects of stimulation of the peripheral vagi. Stimulation of the peripheral vagus (right or left) inhibits the tonus of the esophagus. In preparations in good condition all types and strengths of stimulation applied to vagus nerves cause inhibition so that on the basis of results secured by direct stimulation of the vagus nerves one might conclude that these nerves carry only inhibitory fibers to the circular musculature of the esophagus and cardia. The recovery of the original tonus following the vagus stimulation is usually very gradual (5 to 15 minutes).

Typical tracings illustrating this inhibition are reproduced in figures 3 and 4. In these experiments simultaneous tracings were taken from esophagus and stomach. Tetanization of the vagi with very weak current inhibits the esophagus and may inhibit the stomach (fig. 3). Stronger tetanization of the vagus causes contraction of the stomach parallel with marked inhibition of the esophagus (fig. 4). It will be seen in figure 4 that the esophagus inhibition lasts much longer than the gastric contraction. The esophagus inhibition can be counteracted by intravenous injection of adrenalin (fig. 4, *x*). The vagus inhibitory fibers to the esophagus retain their activity after administration of this drug while the gastric motor fibers of the vagus system are paralyzed.

The inhibition of the stomach by weak tetanization of the vagi confirms the recent findings of Bercovitz and Rogers.

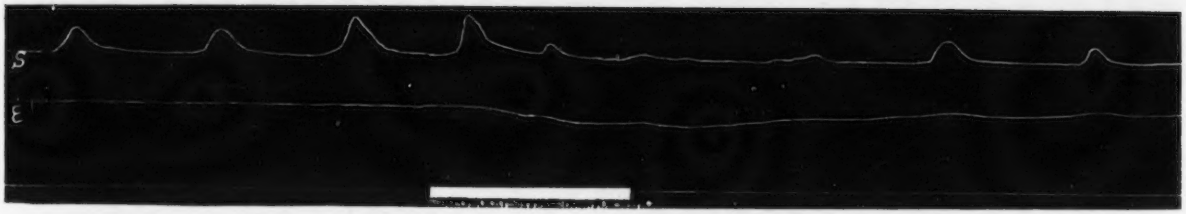

Fig. 3. Turtle, brain pithed. Record of gastric contractions, *s*, and of esophageal tonus, *e*. Signal, tetanization of the left vagus with a very weak current. Showing primary inhibition of the gastric contractions and esophageal tonus by weak stimulation of the vagi.

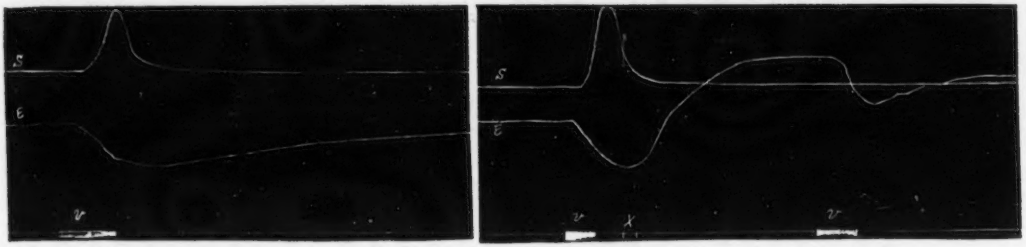

Fig. 4. Turtle, brain pithed. Stomach quiescent, esophagus in hypertonus. Record of gastric contractions, *s*, and esophagus inhibition *e*, on tetanization of the vagi, *v*; *x*, intravenous injection of 10 cc. 1–100,000 adrenalin, showing stimulation of the esophagus by this drug parallel with the paralysis of the gastric motor fibers of the vagi.

In one preparation we secured what seemed to be primary contraction of the esophagus on tetanization of the esophagus. This animal was in very poor condition, in fact, moribund, at the time of preparation. This reaction was obtained even after removal of the esophagus (with the vagi) from the body so that it is evidently due to contraction in the esophagus itself (fig. 5).

The tetanization of the vagi sufficiently strong to cause contraction of the stomach induces the same contraction of the longitudinal musculature of the lower end of the esophagus ($1\frac{1}{2}$ to 2 cm. above and including the cardia) so that if the esophageal balloon extend down to the cardia, the graphic record represents the algebraic sum of the inhibition of the upper two-thirds of the esophagus (circular musculature) and contrac-

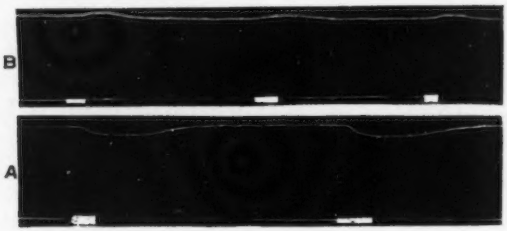

Fig. 5. Turtle. Records from the excised esophagus showing effect of the tetanization of the vagi. *A*, esophagus from an animal in good condition, showing the usual inhibition of esophageal tonus on vagus stimulation. *B*, esophagus from an animal in poor condition (moribund), showing slight increase in tonus from vagus stimulation.

tion of the longitudinal musculature of the lower end of the esophagus, the inhibitory effects predominating.

The results so far show conclusively that the action of the vagi on turtle's esophagus is predominantly, if not exclusively, inhibitory; on the stomach it is predominantly but not exclusively motor. It would thus seem that the hypertonus of the esophagus (circular musculature) following section of the vagi or pithing of the medulla, represents a peripheral automatism normally kept in check by tonic impulses over the vagus nerves, a condition identical with the motor mechanism of the amphibian lung.

We have so far been unable to influence the esophagus by stimulation of the cervical sympathetic nerves, either central or peripheral ends. As in our preparations the entire spinal cord below the medulla was

pithed, no observations could be made on reflex control of the esophagus-vagi inhibitory mechanism, except from the intact cranial nerves. In several preparations with intact medulla and vagi nerves we stimulated the central end of the pulmonary branches of the vagi. This stimulation caused at times dilatation, at times contraction of the esophagus.

3. The action of atropin, nicotin and pilocarpin on the esophagus, and on the vagi fibers to the esophagus. The primary object of these experiments with drugs was to endeavor to separate the inhibitory nervous mechanism from the possible motor nervous mechanism in the manner that proved feasible for the amphibian lung. It will be recalled that in the frog nicotin paralyzes the inhibitory vagi fibers to the lungs, leaving the motor pulmonary vagi functional, so that the pure lung inhibition following vagus stimulation is changed to pure lung contraction after nicotinization. Because of the similarity in origin and neuromuscular structure of the lung and the esophagus, we might look for a similar selective action of nicotin on the vagus system of the esophagus. Support for such selective action is found in the report of Langley on the rabbit that atropin depresses the vagi motor fibers to the cardia to a much greater extent than the inhibitory fibers to that after nicotinization vagus stimulation produces only inhibition of the cardia. Furthermore, Bercovitz and Rogers report on the turtle that atropin paralyzes the gastric motor system of the vagus but not the gastric inhibitory system.

All our experiments with drugs were made on preparations after section of both vagi, or pithing of the medulla, so as to avoid complications from direct action on or reflex action through the medulla. But this means that the esophagus was invariably hypertonic, the degree of hypertonicity varying in different preparations. In most of the experiments in this group parallel records were taken of the gastric and the esophageal contractions to bring out the antagonistic action of the drugs on these two organs.

Atropin, even in very large doses, does not change the action of the vagi on the esophagus. The inhibitory fibers of the vagi are not paralyzed (fig. 6,*B*; fig. 7, *A*, *B*). Because of the fact that atropin usually causes a marked and lasting depression of the esophageal tonus the vagus stimulation prior to atropinization may cause a relatively greater inhibition. But this is no indication of even partial paralysis of the inhibitory nerve fibers.

Nicotin invariably causes a prolonged depression of the esophageal tonus without subsequent stimulation (fig. 8, *A*, *B*). This inhibitory

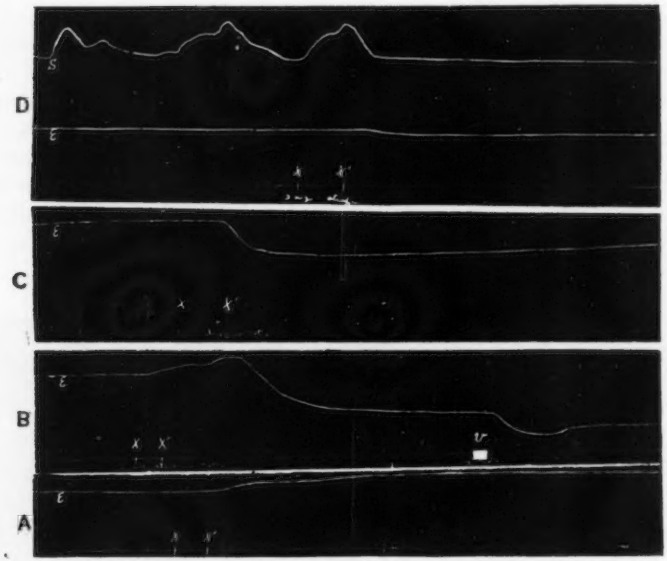

Fig. 6. Turtle, both vagi sectioned. Record of gastric contractions, *s*, and tonus of esophagus, *e*; *x*-*x'*, intravenous injection of atropin sulphate in Ringer's solution. *A*, 1 mgm.; *B*, 2 mgm.; *C*, 1 mgm.; *D*, 2 mgm.; *v*, tetanization of peripheral end of right vagus. Showing primary stimulation, *A*, *B*, and primary inhibition, *C*, *D* of esophageal tonus by atropin; failure of atropin to paralyze the esophageal vagus (inhibitory fibers); primary inhibition of gastric contractions by atropin, *D*.

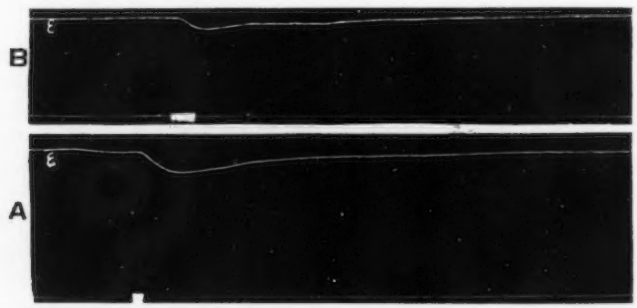

Fig. 7. Turtle. Record of tonus of the esophagus. Signal, tetanization of peripheral end of vagus (*A*, left vagus; *B*, right vagus). *A*, after intravenous injections of 15 mgm. atropin sulphate and 10 mgm. nicotin. *B*, after intravenous injection of 15 mgm. nicotin. Showing failure of atropin and nicotin to paralyze the vagi inhibitory fibers to the esophagus.

action of nicotin parallels so closely that caused by the stimulation of the vagi as to suggest that nicotin acts primarily by stimulating the local vagus mechanism in the esophagus, as is the case in the heart. But there is no further parallel between the nicotin action on the esophagus and the heart, as this drug fails to paralyze the vagi inhibitory fibers to the esophagus (fig. 7, *B*). We may also point out that nicotin has the same primary action (inhibition) on the amphibian lung as on the reptilian esophagus but this drug tends at the same time to paralyze the inhibitory fibers in the pulmonary vagi, thus bringing the lung in line with the heart.

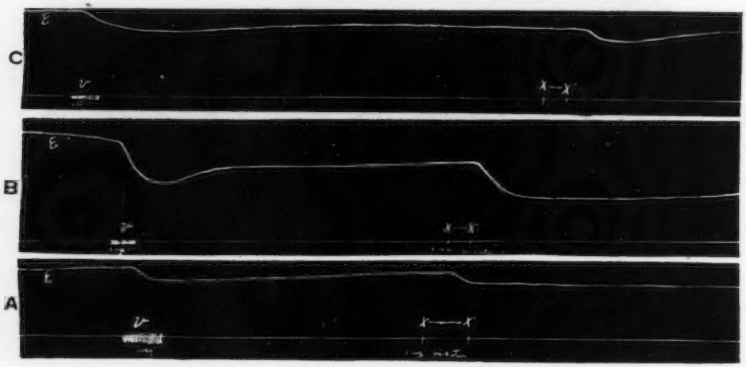

Fig. 8. Turtle, both vagi sectioned. Record of tonus of the esophagus, *e; v*, tetanization of the vagi. *A*, *x-x'*, intravenous injection of 1 mgm. nicotin in 10 cc. Ringer's solution. *B*, *x-x'*, intravenous injection 2 mgm. nicotin. *C*, *x-x*, 10 cc. 1–100,000 histamine hydrochloride. Showing parallel action (inhibition) of vagus, nicotin and histamine.

Nicotin has a primary stimulating action on the turtle's stomach, the stimulation being followed by paralysis. Typical tracings showing the antagonistic action of this drug on the esophagus and the stomach are reproduced in figure 9. The nicotin inhibition of the esophagus is in evidence even after large doses of atropin.

Pilocarpin has a slight inhibitory action on the esophagus, parallel with its stimulating action on the stomach (fig. 10). In no instance did we note any stimulating action of this drug on the esophagus. The stimulation of the motor mechanism of the stomach is the typical action of this drug on the gut, the turtle's esophagus failing to comply with the law.

5. The action of adrenalin and histamine. Adrenalin causes prolonged (30 to 120 minutes) hypertonus of the esophagus (fig. 11). This is true of all concentrations of the drug that are capable of influencing

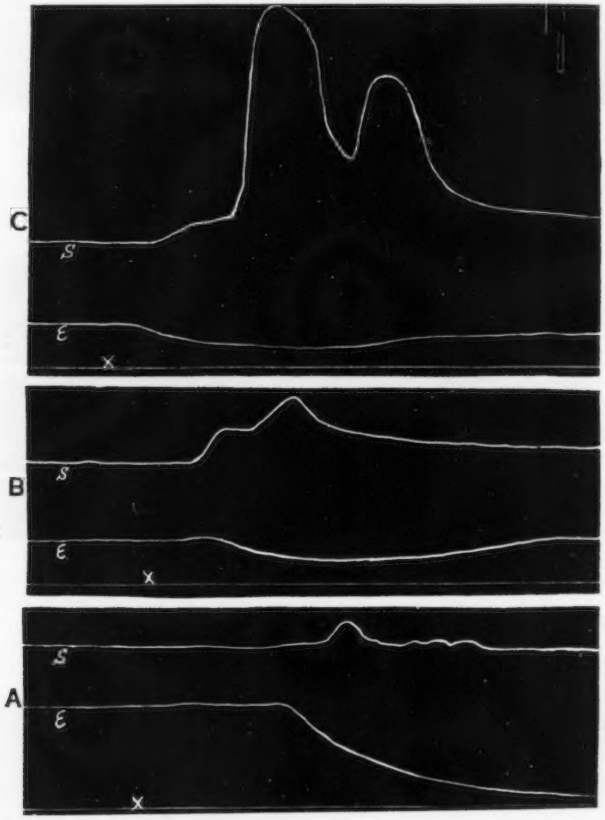

Fig. 9. Turtle, brain pithed. Record of gastric, *s*, and esophageal, *e*, tonus; *x*, intravenous injection of nicotin in 10 cc. Ringer's solution. *A*, 1 mgm. nicotin; *B*, 2 mgm. nicotin; *C*, 2 mgm. nicotin. Showing primary stimulation of the stomach and primary inhibition of the esophagus by nicotin.

this organ. The stimulating action is in evidence even when the esophagus is in marked tonus at the time of intravenous injection of the drug. The action of adrenalin is not influenced by previous adminis-

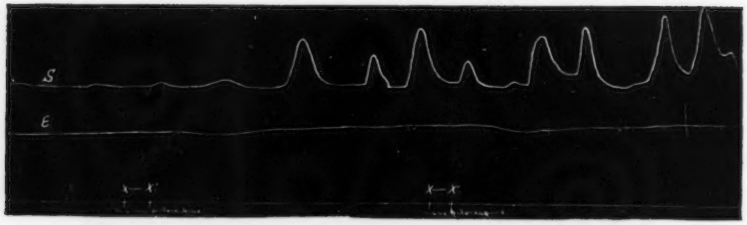

Fig. 10. Turtle. Brain pithed. Parallel record of gastric contractions, s, and esophageal tonus, e; x-x' intravenous injection of. 0.6 mgm. pilocarpin in 10 cc. Ringer's solution. Showing primary inhibition of the esophagus by this drug parallel with the stimulation of the stomach.

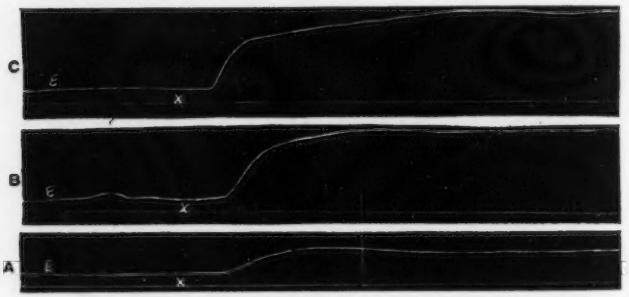

Fig. 11. Turtle, brain pithed. Record of tonus of esophagus, E, x, intravenous injection of adrenalin in 10 cc. Ringer solution. A, adrenalin 1–1,000,000; B, 1–500,000; C, 1–100,000. Showing primary and prolonged stimulation of the esophagus by adrenalin.

Fig. 12. Turtle, brain pithed. Record of gastric contractions, s; L, V, tetanization of left vagus; R, V, right vagus; x-x', intravenous injection of 10 cc. 1–100,000 adrenalin in Ringer's solution. There is an interval of 10 minutes between the vagi stimulations at ↑, preparation left for 12 hours. Showing prolonged paralysis of the gastric motor fibers of the vagi by adrenalin.

tration of atropin or nicotin. Adrenalin does not paralyze the vagi inhibitory fibers to the esophagus (fig. 4).

On the turtle's stomach adrenalin has the typical gut action, that is, inhibition. Adrenalin not only depresses gastric automatism and tonus, but it causes a prolonged paralysis of the gastric motor fibers of the vagus system (fig. 12).

The uniform stimulating action of adrenalin on the esophagus was unexpected, in view of the usual inhibition of the gut by this drug, and in view of the predominating, if not sole, *inhibitory innervation* of the esophagus. We have so far failed to influence the esophagus by stimulation of the cervical sympathetic nerve (central and peripheral end). If there are motor fibers to the circular musculature of the turtle's esophagus, they appear to belong to the vagus system, on which adrenalin is not supposed to act. If we accept the usual view that adrenalin action on muscle mechanisms is *ipse facto* evidence of nervous mechanism having similar action our results prove the presence of motor fibers to the esophagus, but the evidence that these motor fibers belong to the vagus sytem is at variance with the sympathomimetic theory.

We have pointed out the parallel between the reptilian esophagus and the turtle lung in the presence of a peripheral local automatism normally held in inhibitory check by the vagi nerves, and the prolonged hypertonus of these organs caused by section of the vagi. But the parallel fails in the case of the primary action of adrenalin which is inhibitory on the amphibian lung and stimulating on the reptilian esophagus.

Histamine is usually regarded as a universal stimulant of smooth muscle. On the turtle's esophagus histamine has a moderate inhibitory action (fig. 8, *c*).

Stimulation of this organ by histamine was never obtained. But the turtle's stomach is stimulated (feebly) by histamine, the turtle's lung is very strongly stimulated. Histamine stimulates the lung musculature of the frog, but inhibits the lung of the salamanders (21), unless the lung inhibitory nervous mechanism is previously paralyzed by nicotin, in which case the histamine inhibition is changed to stimulation (20).

RESULTS ON FROGS

The effect of ligation of the vagus nerves on the motility of the esophagus and cardiac portion of the stomach. If the vagus nerves are ligated in the neck of a frog whose stomach and esophagus are attached by means of a hook and cord to the short arm of a delicate lever recording on the smoked surface of a slowly moving kymograph, the usual and immediate

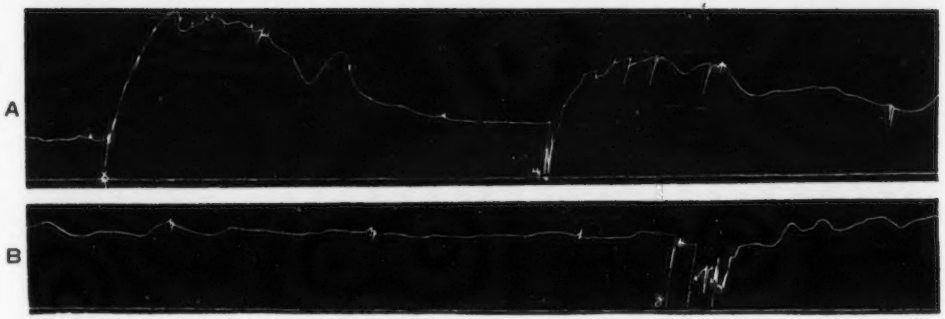

Fig. 13. Graphic record of the esophageal and, in part, gastric movements of the frog. No decerebration. Spinal cord transected below the medulla and pithed. Animal fastened on its back leaving abdominal viscera exposed. Stomach transected 0.5 cm. from cardia and upper portion attached to delicate recording lever writing on a smoked surface. Lifting ligatures under both vagi. Upper jaw cut off exposing anterior ends of cerebral hemispheres.

A, at *x*, ligation of right vagus; at *y*, ligation of left vagus.

B is continuation of *A*. At *z*, brain pithed with wire through opening in anterior end of cranial cavity.

Indicating an escape of the esophagus and stomach from the tonic inhibitory control of the vagus centers.

effect is a sharp contraction of the longitudinal musculature of the esophagus as is recorded at x in A of fig. 13. Following this initial contraction peristaltic waves pass continuously toward the cardia which may pass over the stomach but usually stop on reaching the cardiac sphincter. These peristaltic waves appearing in the lower end of the esophagus and stomach give rise to the irregular undulation seen in the figure just referred to. The motility then gradually diminishes until the remaining vagus nerve is sectioned as shown at y in A of the figure. Following the physiological division of this nerve from the medullary center the tonus remains lightly above that which existed prior to section of the vagi although this does not obtain invariably in the frogs with which we worked. The subsequent destruction of the medulla at z of B in figure 13 has no appreciable effect in raising or lowering the tone of the esophagus or stomach.

From an examination of this figure it would appear that a division of the vagus nerves relieved, especially the esophagus, from the tonic inhibitory influence of the medullary centers. But results are obtained quite frequently in frogs which would seem to be open to but a single interpretation, namely, that the ligation of the vagus nerves acted solely as a mechanical stimulus to the nerves and that the increased tonus and hypermotility resulting therefrom were due solely to marked after-discharge of motor impulses which, impinging on the ganglion cells of the peripheral automatic mechanisms, continue to send out discharges to the smooth musculature for an appreciable time after the direct stimulation of the nerve fibers in the neck has ceased. This interpretation is rendered quite probable for one of us has shown that the quiescent heart of molluscs can be induced to beat rhythmically for some time following electrical stimulation of the motor heart nerves (5).

Figures 14, 15 and 16, representing essentially the same conditions are offered as evidence in support of this interpretation. In figure 14, A, the right vagus nerve was ligated in the neck with the usual hypertonus and motility of the esophagus. In this animal the medulla had been pithed $1\frac{1}{2}$ hours before taking this record. The response can be accounted for solely on the basis of mechanical stimulation of the motor fibers of the vagus by the tightening of the ligature. In this instance the hypertonus was only transient. Eighty minutes later, electrical stimulation with a tetanizing current (fig. 14,B, at y) induced an activity of the esophagus quite similar to the previous ligation of the vagus in the neck. Figure 15 records a similar experiment and suggests a similar interpretation. It is reproduced solely because the pseudo-hypertonus

on ligation of the vagus (at x in A) and stimulation of the peripheral end (at y in B) records only esophageal movements since the hook attached to the recording lever was placed *under* the lower end of the esophagus.

As a final example of what appears to be a simple mechanical stimulation of the vagus nerve on mechanical division of it in the neck we offer the experiment recorded in figure 16. Here the left vagus was ligated at x in A, with prolonged motor activity of esophagus (and stomach) while in markedly hypertonic state. Simply placing a ligature under the right vagus with the incidental stimulation of the nerve (B at y)

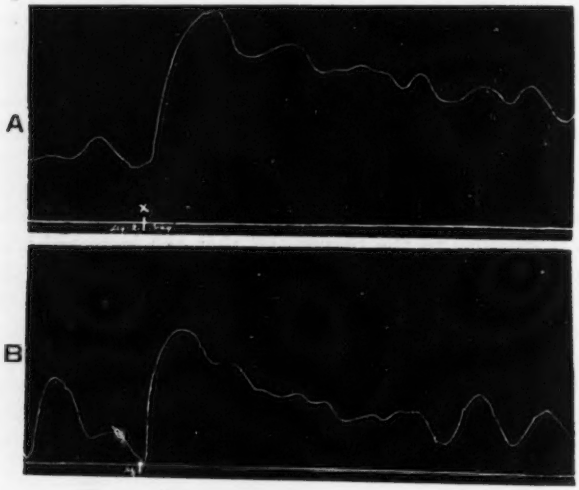

Fig. 14. Graphic record of esophageal movements of a frog. Frog decerebrated, upper jaw cut off exposing anterior ends of cranial cavity. Animal laid on back and virtually eviscerated, the cardiac end of the stomach and entire esophagus alone preserved. The cardiac end of stomach attached to delicate lever. Medulla pithed without striking effects on motility of esophagus. A, record taken 2 hours after beginning of experiment, and about 1½ hour after destruction of the medulla. At x, ligation of the right vagus in the neck. B, at y, electrical stimulation of the right vagus for 2 seconds with a tetanizing current of moderate strength 80 minutes after ligation of the nerve in A. Indicating that the hypertonus of the esophagus following ligation of the vagi in the neck under conditions where these nerves are still in communication with the vagus center is due at least in part to prolonged stimulation of the peripheral automic mechanism by the mechanical stimulation of the motor fibers by the ligature and not an escape of esophagus from tonic central inhibition.

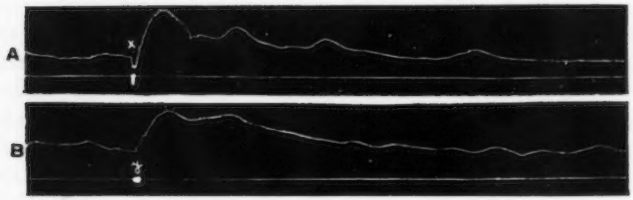

Fig. 15. Graphic record of the esophageal movements of a frog. Frog decerebrated. Spinal cord transected below the medulla and pithed. Animal fastened on back and abdominal and thoracic contents exposed. Hook *under* lower end of esophagus, attached to delicate recording lever. *A*, at *x*, ligation of one vagus. *B*, at *y*, electrical stimulation of the same vagus with a tetanizing current of moderate strength. Showing marked similarity on the esophageal movements between ligation and electrical stimulation of the same nerve.

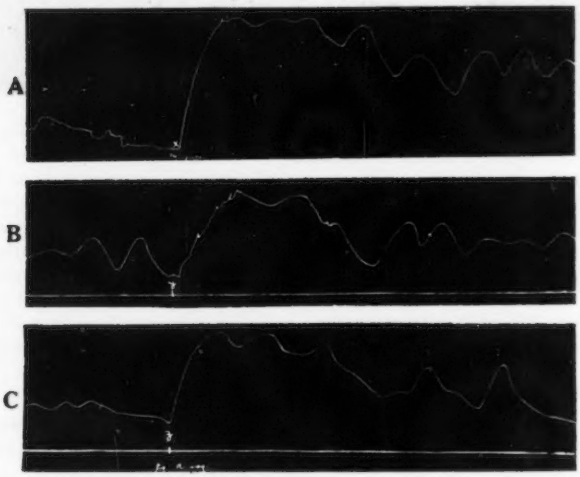

Fig. 16. Graphic record of the movement of the esophagus of a frog. Frog decerebrated. Spinal cord transected below the medulla and pithed. Stomach transected 0.5 cm. from cardia and attached at that point to delicate lever. *A*, at *x*, ligation of left vagus nerve in the neck. *B*, at *y*, careful introduction of a ligature under the right vagus 50 minutes after *A*. *C*, at *z*, ligation of right vagus 20 minutes after *B*. Showing in *B* the similarity in movements of the esophagus following mechanical stimulation of the nerve with ligation of the nerves (*A* and *C*) still connected with the medullary centers.

had a similar effect. The ligation of this nerve subsequently at z, in C, caused a motor response in the esophagus similar to the ligation of the first vagus nerve prior to its physiological division. It is furthermore plain that the hypertonus and motor activities are but of short duration.

The effect of sudden destruction of the medulla by chloroform. To eliminate as far as possible direct stimulation of the motor fibers of the vagus directly by ligation or indirectly by pithing of the medulla with a wire, we performed several experiments in which we destroyed the medullary centers suddenly and completely by injecting chloroform directly into them. Assuming that under these conditions the vagus centers would be rapidly destroyed without experiencing a temporary

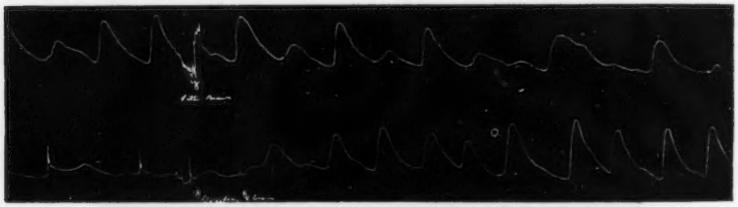

Fig. 17. Graphic record of the esophageal movement in the frog. Frog decerebrated. Spinal cord transected below the medulla and pithed. Upper jaw cut away just anterior to the eyes thus exposing anterior ends of cerebral cavity. Animal on back. Stomach attached near cardia to delicate lever writing on smoked surface. At x, intramedullary injection of one drop of chloroform through opening in anterior end of cranial cavity; at y, pithing medulla with a wire through the same orifice. Indicating an escape of the esophagus (and stomach) from the tonic inhibitory control of the vagus centers by destroying these centers suddenly with chloroform at x, for subsequent pithing of centers at y, had no further effect on the rhythm initiated by the chloroform destruction.

stimulation, all motor effects resulting from the destruction would indicate release of the esophagus and stomach from the normal tonic inhibitory control exerted by them.

Figure 17 gives the graphic results of such an experiment. Here (lower line) both the esophagus and stomach were relatively quiescent to begin with, the sharp upstroke prior to x representing movements of the head and neck of the animal. Immediately following the destruction of the medullary centers with chloroform at x, a pronounced rhythm appeared particularly in the esophagus which persisted throughout the period of experimentation. The subsequent mechanical maceration of the medulla with a pithing needle at y (upper line) had no further

effect. Experiments of this type indicate that the vagus centers exercise a tonic inhibitory control over the stomach and especially the lower end of the esophagus.

3. Changes in outline of the esophagus and stomach following ligation of the vagus nerves in the neck. The methods used for the graphic record of the stomach and esophagus following division of the vagi or destruction of the medullary centers involve tension on these structures. It seemed desirable, therefore, to show that the results recorded above transpired when the esophagus and stomach were resting in their normal positions within the abdominal cavity without the disturbing factors of diminished blood supply and traction on these structures. By a method previously described we found it possible to sketch rapidly the outlines of these viscera and thus obtain a consecutive record of the movements following ligation of the vagus nerve which would mean more to the reader than any amount of description.

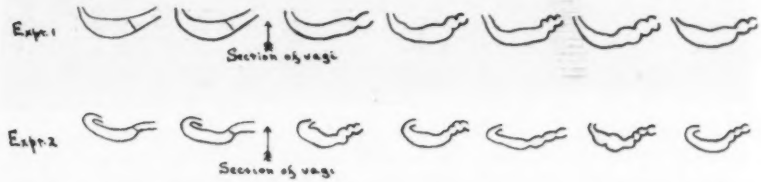

Fig. 18. Frog. Tracings of the outline of the esophagus and stomach before and after section of the vagus nerves. Showing hypermotility of the esophagus following isolation from the central nervous system.

Two experiments of this type follow:

Experiment 1. February 3 (fig. 18). Frog decerebrated and spinal cord pithed below the medulla. Animal fastened on its back. Thoracic and abdominal viscera exposed. Left lung excised. Ligatures under both vagi. Sketches of the esophagus and stomach made on a ground glass plate. Interval between sketches about 5 seconds.

Immediately following the ligation of the vagus nerve the contour of the quiescent esophagus changed. The esophagus shortened. Peristaltic waves swept over it for about 45 minutes. Occasionally the peristaltic waves passed over the stomach. The appearance of the esophagus and stomach immediately before and after ligation of the vagus nerves is shown in the upper series of sketches of figure 18.

In the second experiment the animal was prepared in a similar manner. In this animal, however, we excised the heart, lungs and liver. In

this experiment the esophagus did not shorten especially following ligation of the vagus nerves. On the other hand, peristalsis started promptly and continued throughout the period of observation (about 35 minutes). Sketches shown in lower line of figure 18 note the changes in the esophagus and stomach which followed ligation of the vagus nerves.

4. Incomplete tetanus of the esophagus and stomach on stimulation of the peripheral end of the vagus nerve with a tetanizing current. Figure 19 is offered as an example possibly of incomplete tetanus of the esophagus on electrical stimulation of the vagus nerve. The prolonged stimulation of the vagus at *y* leads to a prolonged hypertonic state of the esophagus on which were superimposed the individual contractions of automatic

Fig. 19. Graphic record of the esophageal and, in part, gastric movements of the frog. Frog decerebrated. Spinal cord transected below medulla and pithed. Animal fastened on its back. Abdominal and thoracic viscera exposed. Stomach transected 0.5 cm. from the cardia and upper end attached to delicate lever writing on a smoked surface. Both vagi sectioned. *x*, stimulation of peripheral end of vagus with tetanizing current of moderate intensity. *y*, prolonged stimulation of the same vagus with the same strength of current. Showing incomplete tetanus of the esophagus following prolonged vagus stimulation.

rhythm. In view of the fact, however, that a very brief period of stimulation can produce a similar contracted state of the esophageal musculature lasting for some time after cessation of the stimulation, it is not so certain that the prolonged period of stimulation is responsible for the result which was obtained in this instance.

5. Effect of section and stimulation of the cervical sympathetic and splanchnic nerves on the motility of the esophagus and stomach. The results of section and stimulation of the cervical sympathetic had no pronounced effects on the movement of the esophagus and the stomach. A similar statement applies to our experience with stimulation of the peripheral end of the splanchnics. We attach no positive significance to our negative results.

6. The action of various drugs on the neuro-muscular mechanism in the esophagus of the frog: Nicotin. We have no extensive series of experiments relative to the action of this drug on the state of activity of the esophagus. Since nicotin abolished the central inhibitory control of vagus on the lung of the amphibia, we injected the drug intravenously in a few frogs to note whether the quiescent esophagus would escape as evidenced by marked shortening and the appearance of peristaltic waves. In 2 mgm. doses this effect was produced but it is obvious on the basis of our results that the increased motility might be due to direct stimulating action of the drug on the peripheral neuro-musculature apparatus. For where the evidence for the presence of inhibitory nerves in the vagi to the esophagus is wanting or doubtful, the description of the escape of the esophagus from their control by the use of nicotin is hardly justified.

Atropin. Since the stimulation of the peripheral end of the vagus invariably gave a marked motor response it seemed possible to paralyze the peripheral terminations of this nerve by suitable doses of atropin. Under such conditions we might be able to demonstrate the presence of inhibitory fibers.

Atropin failed to have the slightest recognizable effect on the peripheral motor terminations of the vagus. Even after giving the animal 10 mgm. of this drug intravenously, stimulation of the peripheral end of the vagus with a tetanizing current sent the esophagus into tetanus (fig. 20 at x).

Since the intravenous injection of the drug was made under conditions of possibly poor circulation through the esophagus, we injected three frogs subcutaneously with 8, 10 and 20 mgm. of the drug one hour before preparing them for graphic registration. In every case the results were the same. One frog which had received 20 mgm. at 9:15 was given another 20 mgm. of the drug subcutaneously at 4:30 p.m. At 4:50 p.m. when the respiration had ceased the animal was prepared for graphic registration. Even before section of the vagi or destruction of the medulla and spinal cord lively movements of the esophagus and stomach were in evidence. Section and stimulation of the vagi gave the usual motor effects. In fact, we were able to tetanize the esophagus by electrical stimulation of the nerve. However the results are interpreted, one thing seems clear, namely, that the motor termination of the vagus cannot be paralyzed by atropin.

Adrenalin. This drug even in dilutions of 1:100,000 exerts, when applied locally to the esophagus, a marked inhibitory effect on the periph-

eral rhythm present in the esophagus as is illustrated in figure 21 at x. Nor is the inhibitory action entirely due to the chloretone content present in the dilute solution of adrenalin used; for irrigation of the esophagus with a chloretone solution of equal concentration as at y of figure 21 produces only a slight and fleeting inhibition.

When the concentration of adrenalin is increased to 1: 10,000 and applied locally the inhibition is even more pronounced and long lasting. Even more remarkable is the fact that during the marked inhibition following irrigation, stimulation of the vagus nerve with any strength of current is without effect as is shown at y and z in A of figure 22. As

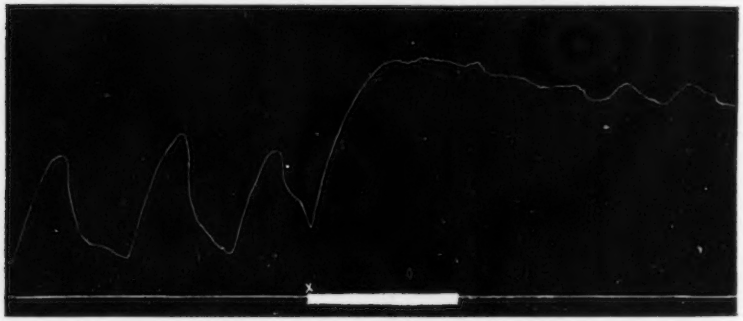

Fig. 20. Graphic record of the peripheral rhythm of the esophagus and stomach of a frog. Animal decerebrated. Spinal cord transected below the medulla and pithed. Both vagi have been sectioned and the peripheral end of one retained for stimulation. Twenty minutes before taking this record the animal received an intravenous injection of 10 mgm. of atropin sulphate through the anterior abdominal vein. At x, stimulation of the peripheral end of the vagus with a tetanizing current of moderate strength. Showing that atropin failed to paralyze the peripheral motor terminations of the vagus.

the action of the adrenalin wears off stimulation of the vagus nerve gives the usual motor effect. This is shown in B of figure 22, where the electrical stimulation of the vagus, x, for 3 seconds was promptly followed by motor effect. The irrigation of the esophagus at y with adrenalin led to an inhibition during which an electrical stimulation at x gave only a feeble motor response.

From this it would appear that adrenalin not only abolishes a peripheral rhythm of the esophagus however induced but, when administered in sufficient concentration, paralyzes temporarily the motor terminations of the vagus.

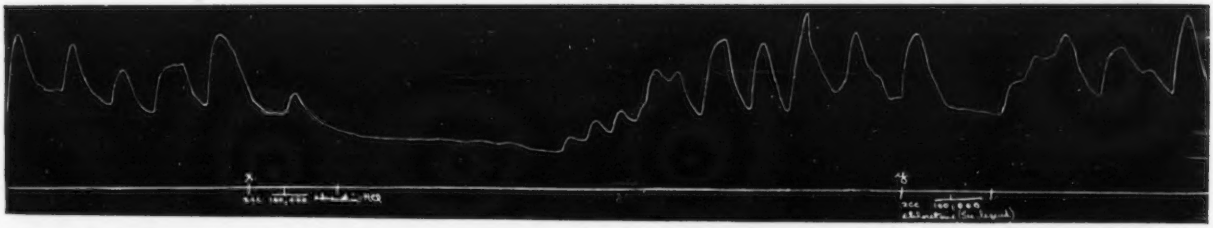

Fig. 21. Graphic record of the esophageal movements of a frog. Frog decerebrated. Spina' cord transected below the medulla and pithed. Vagi sectioned and medulla destroyed by pithing. Stomach transected 0.5 cm. from the cardia and attached at that point to delicate lever writing on smoked surface. *x*, irrigation of the esophagus with 2 cc. 1:100,000 adrenalin chloride so ution during period indicated by the signal magnet. *y*, irrigation of esophagus with 2 cc. of chloretone solution equal in concentration to the concentration of chloretone present in 2 cc. of 1:100,000 adrenalin chloride solution. Showing that the inhibition by the adrenalin chloride solution is not due to its chloretone content.

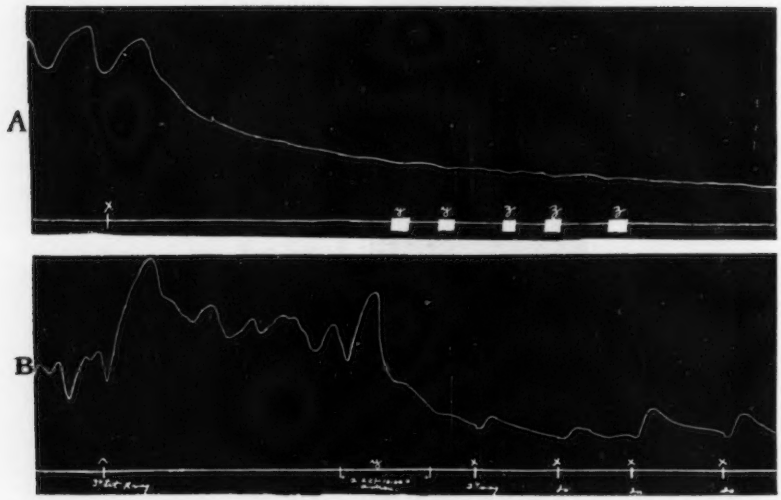

Fig. 22. Graphic record of the esophageal and gastric movements of a frog. Decerebrated. Spinal cord transected below medulla and pithed. Fastened on back and thoracic and abdominal viscera exposed. Both vagi sectioned. Stomach transected 0.5 cm. below cardia and upper end attached to delicate lever. *A*. Animal had received 10 mgm. atropin sulphate through cannula in anterior abdominal vein. This amount failed to paralyze the terminations of the vagus (see fig. 20). At *x*, irrigation of the stomach and esophagus with 2 cc. of 1:10,000 adrenalin chloride solution; at *y*, stimulation of the peripheral end of the vagus with weak tetanizing current; at *z* stimulation of the vagus with a strong tetanizing current. Showing adrenalin inhibition of the peripheral rhythm of the esophagus and stomach as well as paralysis of the motor termination of the vagus by this drug. *B*, at *x*, electrical stimulation of the right peripheral end of the vagus for 3 seconds with a tetanizing current of moderate intensity before and after the irrigation of the esophagus and stomach at *y* with 2 cc. 1:10,000 adrenalin chloride solution. Showing motor effects on electrical stimulation of the vagus, adrenalin inhibition of the esophageal and gastric rhythm and partial paralysis of the motor terminations of the vagus by adrenalin with beginning recovery.

DISCUSSION OF THE RESULTS

The results on the turtle are clear cut and convincing. There can be no doubt that the local motor automatism of the esophagus is held in tonic inhibitory check by the vagi, so that on section of the vagi, or destruction of the medulla, the circular musculature of the esophagus goes into hypertonus lasting throughout the experiment. In

line with this, stimulation of the peripheral vagus inhibits the circular musculature, including the cardia. This type of motor control of the reptilian esophagus and cardia is identical with the motor control of the amphibian lung.

It is true that extensive dissection is required in the turtle to permit satisfactory experiments of this type but we cannot see how the trauma can account for any essential part of our results. It seems more likely that the trauma to the nervous system depresses the medullary centers so that the hypertonus of the esophagus following section of the vagi is actually less than normal. The esophageal hypertonus is not due to local stimulation of the esophagus by the slightly inflated balloon, as the empty esophagus goes into hypertonus on vagi section.

The results on the frog cannot with certainty be interpreted as showing a predominating vagal inhibition of a local esophageal automatism. There is a remarkably prolonged motor effect on the esophagus of momentary stimulation of the vagus, a factor not recognized by Goltz and his followers. On the other hand, the marked inhibitory action of the drug adrenalin may be interpreted as demonstrating an inhibitory nervous mechanism. Our failure to demonstrate extrinsic inhibitory nerves in the vagi, cervical sympathetic or splanchnics, by section or by stimulation, may be due in part to the condition of the frog. The work was done during the winter months and the animals were not in the best of condition. But the temperature itself was not a factor, as some of the animals were slowly warmed up to room temperature before the experiment.

The reader may recall that in frogs in poor physiological condition the tetanus of the lungs, so marked in good preparations, may be insignificant or altogether absent. It seems reasonable to suppose that the inhibitory mechanism for the esophagus would be similarly depressed by poor condition of the animals, and hence to be demonstrated only in the very best of preparations. Peripheral "shock" may also be a greater factor in the esophagus than in the lung. It should be noted, however, that the frog with section of both vagi swallows air into the stomach, but cannot force the air into the contracted lungs (Patterson). This would seem to indicate that the hypertonus of the isolated lung is greater or of longer duration than the hypertonus of the esophagus isolated from the medulla.

Contrary to our results on the amphibian lung and the indications in the literature on mammals, neither atropin nor nicotin were of any service in differentiating between motor and inhibitory nervous mechan-

isms to the esophagus in frogs and turtles. These drugs do not paralyze the vagus motor fibers to the frog's esophagus or the vagus inhibitory fibers to the turtle's esophagus. Adrenalin paralyzes the gastric motor fibers of the vagi both in the turtle and the frog, but not the vagi inhibitory fibers to the turtle esophagus.

TABLE 1

Primary action of vagi section, of vagi stimulation, and of certain physiologically important drugs on the motor mechanism of stomach, esophagus, lungs and heart. Stimulation or increased action = +; inhibition = −. When both augmentation and inhibition may result the predominating action is placed above or first

	TURTLE				FROG				SALAMANDER	
	Stomach	Esophagus	Lung	Heart	Stomach	Esophagus	Lung	Heart	Lung	Heart
Section of vagi	+a −	+a −?	+?	+a	+a −	+a	+a	+a	+a	+a
Stimulation of vagi	+ −	− +?	+	− +*	+ −?	+ −?	− +*	− +*	−	−
Adrenalin	−	+	−	+	−	−	−	+	−	+
Histamine	+	−	+	−	−	−	+	−	− +*	−
Nicotin	+	−	+	−	?	?	−	−	−	−
Atropin	−	+ −	−	+	?	?	?	+	?	?
Pilocarpin	+	−	+	−	+	+	?	−	?	?

a, escape from inhibitory control of vagi. * after nicotin.

The striking lack of parallel between vagi and drug action on stomach, esophagus, lungs and heart may be recapitulated in tabular form (table 1).

The present results strengthen the view that *the primary action of many drugs on visceral motor mechanism depends on the predominant type of innervation (motor or inhibitory) of these organs.* The action of adrenalin on the turtle's esophagus appears, however, to run contrary to this view, as the predominating vagus influence is inhibitory, but adrenalin nevertheless augments the esophageal tonus.

On examining this table carefully it will be noted that histamine, nicotin and pilocarpin have an action on the stomach, esophagus, lung and heart of turtle directly opposite to the action of adrenalin and atropin. This direct antagonism of the drugs mentioned was particularly striking between adrenalin and pilocarpin.

The present results on the turtle's esophagus, together with the indications in the literature of at least temporary hypertonus of esophagus and cardia in some mammals following section of the vagi, show that the factors of local automatism and extrinsic inhibitory nerves must be taken into account in motor disturbances of esophagus and cardia. But experimental results on one species cannot, without further consideration, be transferred to another species, because of the evident variations in the degree of primitive motor control retained by this end of the gut (including the lung) in different animal groups.

SUMMARY

Turtles: 1. Section of the vagi or pithing of the medulla leads to hypertonus of the esophagus (circular musculature) and cardia. Stimulation of the vagi causes inhibition of the esophagus (circular musculature) and cardia. The predominant vagus innervation of the esophagus is therefore inhibitory, and this mechanism is in tonic activity.

2. The observations of Bercovitz and Rogers that section of the vagi may induce hypertonus and initiate contractions of the stomach, and that stimulation of the vagi causes inhibition of the stomach are confirmed, but the predominating action of the gastric vagi is motor.

3. Atropin and nicotin do not paralyze the vagi inhibitory fibers to the esophagus.

4. Adrenalin stimulates the esophagus, inhibits the stomach and paralyzes the gastric motor fibers of the vagi. The drug does not paralyze the vagi inhibitory fibers to the esophagus.

5. Nicotin, atropin, histamine and pilocarpin have opposite actions on the stomach and the esophagus.

6. No evidence of sympathetic innervation of the esophagus was obtained.

Frogs: 1. Section of the vagi causes in frogs a hypermotility of the esophagus particularly, but to some extent also of the stomach. Our evidence would seem to indicate that this hypermotility is due in part to an escape of these structures from the tonic inhibitory control of the medullary centers but essentially to the mechanical stimulation of the motor fibers carried by the vagi to these structures.

Stimulation of the peripheral end of the vagus nerves give invariably pronounced motor effects on the esophagus and stomach. An incomplete tetanus of the esophagus may result from such stimulation.

We could secure no direct evidence that the vagus and cervical sympathetic nerves carry inhibitory fibers to the esophagus and stomach of the frog. No positive significance is attached to these negative findings.

2. In the frog atropin even when used in large doses fails to paralyze the motor termination of the vagus nerves.

Adrenalin, on the other hand, promptly inhibits the peripheral automatic activity of the esophagus and stomach. This drug likewise temporarily paralyzes the motor termination of the vagus.

General. 1. The present data afford additional evidence that the primary action of many drugs on visceral motor mechanisms depends on the predominant innervation (motor or inhibitory) of the organs.

2. The present data indicate that tonic inhibitory innervation via the vagus nerves plays a rôle in the motor control of the esophagus and the cardia. But the conditions found in one animal group or species do not necessarily apply to another group or species, as the degree of differentiation in the motor control from the primitive condition appears to vary greatly in different species.

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QUANTITATIVE STUDIES ON INTRACELLULAR RESPIRATION

V. The Nature of the Action of KNC on Paramecium and Planaria, with an Experimental Test of Criticism, and Certain Explanations Offered by Child and Others[1]

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Successful experimentation in physiology generally depends to a very large extent upon the degree to which the process under investigation can be experimentally isolated. One of the means which can be used in this sense, to isolate the process of respiration in protoplasm, is the use of cyanides. It has become a well-known fact that cyanides in general change the rate of oxygen consumption and carbon dioxide production to various degrees depending upon conditions. The effect which is best known is the inhibitory action of potassium cyanide. Little if anything is known about its apparent tendency under certain conditions to accelerate the rate of respiration.

It has sometimes been tacitly assumed if not expressly stated, on the basis of good and extensive, but not complete experimental evidence that *all* biological oxidation processes are inhibited more or less by cyanide. There has been a tendency to assume that because KNC inhibits cell respiration in some or many organisms, therefore it should inhibit the respiration in all types of living protoplasm. See for example Hyman (1) Child and Hyman (2, p. 217).

It was therefore somewhat of a surprise to the writer when he was unable to demonstrate any inhibitory action of KNC on the oxygen consumption of Paramecium (3). These experiments suggested the advisability of measuring the effect of cyanide upon Planaria which had been used by Child and his co-workers in extensive studies in

[1] The writer is indebted to Mr. Emmett Rowles for valuable assistance in many of the experiments in this work.

which solutions of cyanide were used to determine supposed differences in rate of metabolism.

The experiments by Allen (16) showed that a perfectly reversible inhibition amounting to 70 or 80 per cent of the normal oxidation could be obtained. These results were confirmed by Hyman (1).

Since that time numerous papers have appeared in this and other journals by Child (4), (5), Robins and Child (6) and Hyman (7), (8), (9), in which various attempts are made at an explanation of the apparent discrepancy between the experimental results obtained from Paramecium and those from many other cells and organisms typically represented by Planaria. These criticisms and suggested explanations of the results may for convenience be classified under two heads.

First: Criticism of experimental method and interpretation of the data from the experiments on Paramecium. See Hyman (1), Child (5, footnote p. 155).

Second: Attempts by Child (4, p. 255 and footnote, p. 256) at explanations as to how these differences in experimental results between Paramecium and other organisms might come about. Repetitions of these criticisms and explanations have been made in several papers by Child and Hyman at different times; therefore I take it that in the minds of these writers the explanations which they offer constitute more than mere tentative suggestions. It will be the purpose in this paper briefly to answer the criticisms and show that the explanations which have up to the present been offered, can not possibly be correct. In a following paper results of experiments recently completed which give in the opinion of the writer the correct interpretation of the behavior of Paramecium toward cyanides will be presented.

Criticisms of the accuracy of experimental methods. In order that the reader may be able to see in the simplest way the writer's attitude toward the criticisms by Hyman (1, pp. 356–358) and Child (5, footnote p. 155), these specific criticisms are summarized and numbered, and the answer to each one is given briefly as follows.

1. It is stated that the solutions of KNC used by the writer in the tests on Paramecium were too dilute to affect the rate of oxygen consumption since Paramecium is more resistant to KNC than other organisms and dies only in relatively very high concentrations of cyanide. The answer is that a wide range of concentrations was used, as shown in the tables (3). The higher concentrations which were used *did* kill the animals although no trace of inhibition was evident. They were lethal concentrations, and that is all that is necessary in this connection

to prove the point that death in KNC solutions can take place without inhibition of the oxidations.

2. Child and Hyman state that it is probable that the alkalinity of the KNC solutions has a stimulating effect on respiration in Paramecium. This, however, is not correct as will be shown below.

3. It is stated that the oxygen consumption by protozoa is very small, therefore quantitative measurements are difficult to perform. Presumably Hyman means the *rate* of oxidation. The truth of the latter statement has never been shown, in fact the opposite may be true. If her statement means that the total quantities of oxygen consumed by the Paramecia during the experiments are too small to be accurately measured, then the answer is that the quantity consumed varied from about 15 to over 50 per cent of the total oxygen content of the water in the bottle. A glance at the tables will show this. The experimental error in Winkler's method when properly used is, as is well known, about 1 to 2 per cent.

4. The initial effect of KNC might be quite opposite to its final effect and therefore a test of the total oxygen consumption, such as that which was made by the writer in any one experiment, does not give any indication of what the time course of the effect of the cyanide really was. The answer is obviously that the experiments given were never designed to show what is the time course of the effect of cyanide on Paramecium. They do show that the toxic solutions of KNC had no more inhibitory effect than the non-toxic concentrations, which is the point in question. In fact, the toxic solutions had an accelerative effect, if any. This for example is true in the 10-hour period and also in the 29-hour period in table 3 of the experiment.

5. The error due to iodine absorption by the cells was not adequately determined, so that it could not be adequately applied as a correction to the results. The answer is that for the essential purpose of the experiment and conclusions drawn the iodine error may be ignored entirely, for we are primarily comparing the oxygen consumption in low and high concentrations of cyanide. See for example table 3, column 7, in which the iodine error is necessarily the same in all the determinations.

6. To Hyman a very important lack in the experiments is that "no figures are given anywhere of the normal rate of oxygen consumption of the same lots of Paramecia" (p. 357, l. c.). Proof that different 1 cc. samples of the same Paramecium suspension consume identical amounts of oxygen under the same conditions will be found in, for exam-

ple, table 4, column C. Several other experiments in an earlier paper should make this clear to any one (cf. Lund (10)). It is evident from this that in the experiments referred to by Hyman it was not at all necessary to test the oxygen consumption by the same lots of Paramecia.

7. In my paper (3) it was suggested as probable that after cytolysis of Paramecium, those oxidations ceased which especially represented that part of the respiratory exchange of a cell which is directly concerned with transformation of chemical energy into, for example, mechanical work.

TABLE I

Showing the difference in total output of CO_2 by normal Paramecia and by the same number of Paramecia after mechanical disintegration. The latter was a colloidal solution, no cell fragments present. Duration of tests 2½ and 3 hours. Total CO_2 liberated is given in equivalents of cc. n/100 HCl

EXPERIMENT	BOTTLE	CONTROLS		5 CC. DISINTEGRATED PARAMECIA IN TAP WATER
		Blank; 5 cc. tap water; no Paramecia	5 cc. normal Paramecia in tap water	
		cc. n/100 HCl	*cc. n/100 HCl*	*cc. n/100 HCl*
I	1	0.20	0.85	0.21
	2	0.17	0.98	0.22
	3		0.98	0.13
	4			0.20
Average.....		0.18	0.93	0.19
II	1	0.20	1.00	0.29
	2	0.20	1.13	0.35
	3		0.84	0.33
	4			0.29
Average.....		0.20	0.99	0.31

The suggestion was based upon the observations of Warburg (11) on the respiration of mechanically disintegrated fertilized eggs, and of Fletcher and Hopkins (18) on disintegrated muscle. This suggestion, it is maintained by Hyman, is unwarranted. Accordingly, in order to obtain further direct evidence on this point from Paramecium, a few experiments were made in which the rate of CO_2 elimination by identical lots of Paramecia were compared. One of the lots was mechanically disintegrated and the other left normal as a control. The animals were mechanically disintegrated in tap water by means of a small circular knife running at 3000 revolutions per minute. No trace of cell

fragments remained, the suspension was made up of small granules and was colloidal in character. The method for determination of CO_2 was a modification of that described in a former paper (12). Four tests were made, all of which gave the same results; two of these are given in table 1.

It is clear from the results that under the conditions of the experiment the CO_2 elimination in Paramecium practically disappears after destroying the protoplasmic structure of the cell. This fact along with the data given in table 4 (3) strongly supports the suggestion which was originally made, that stopping of the greater part of normal cell respiration follows cytolysis even in the absence of KNC. Similar evidence was obtained when high oxygen concentration was used as a toxic agent (10).

The problem of the relation of protoplasmic structure to cell respiration is, however, in need of extensive and accurate investigation before it will be possible to lay down any general conclusions.

Is the toxic action of KNC solutions on Paramecium due to their high alkalinity? The only criticism made by Hyman and Child, that would seem to have any justification, is that given under criticism no. 2 above, where it was maintained the alkalinity of the cyanide solutions might affect cytolysis and also the rate of respiration in Paramecium.

It would appear possible, since no inhibitory effect on the respiration could be detected in concentrations of KNC from m/27400 up to as high as m/274, and since the animals began to die within 30 hours in m/274, that the high alkalinity was the cause of death rather than the action of cyanide as such. An answer to this question can readily be given by comparing the survival time of identical lots of Paramecia from the same suspension in equimolecular solutions of KNC and KOH, whose hydrogen ion concentrations are known. The following table gives the result of one experiment from among several which were carried out with identical results.

It is clear from the table that the high toxicity of the KNC solutions is not due to their high alkalinity, for the KOH solutions whose pH was even slightly higher than that of the KNC solutions did not kill any of the animals within the duration of the experiment. We must conclude therefore that the toxicity of KNC solutions used in the experiments on the rate of oxidation in Paramecium was not due to the high alkalinity but that the toxic action of the higher concentrations of KNC in which no trace of inhibition of respiration was found, was due to the toxicity of the cyanide as such.

Does the hydrogen ion concentration of the medium have any effect upon the rate of oxygen consumption by Paramecium? It has been suggested by Child (5, footnote, p. 155, and in private communication) that cyanide solutions, due to their high alkalinity, might stimulate the cell to a higher rate of oxidation which therefore might counteract or conceal

TABLE 2

Showing the relative survival times of Paramecium caudatum in the same concentrations of KNC and KOH. The table also shows the absence of a relation between survival times and pH of the solutions. Volume of each solution 100 cc. pH of tap water = 8.2. x = few dead. xx = many dead. xxx = most dead. 0 = all dead

CUBIC CENTIMETERS OF M/10 KNC TO 100 CC. TAP H_2O	pH	TIME IN HOURS												
		0	3	7	23	25	29	37	59	71	83	107	134	153
0.2	8.2							x	xx	xx	xx	xxx	xxx	0
0.4	8.4							x	xx	xx	xxx	xxx	xxx	0
0.6	8.8							x	xx	xxx	xxx	xxx	xxx	0
0.8	9.0						x	xx	xxx	xxx	xxx	0	0	0
1.0	9.2					x	xx	xxx	0	0	0	0	0	0
2.0	9.4				x	xx	xxx	0	0	0	0	0	0	0
CUBIC CENTIMETERS OF M/10 KOH TO 100 CC. TAP H_2O														
0.2	8.4													
0.4	8.6													
0.6	8.9										All living and normal			
0.8	9.2													
1.0	9.4													
2.0	9.6													

the characteristic inhibitory effect of the cyanide. While this might appear at first sight a plausible explanation of the apparent absence of inhibition of the rate of oxidation in Paramecium, after a little reflection upon the striking uniformity of the numerical values, given for example, in column 7 of table 3 (3), it becomes evident that it would be

a strange coincidence if in concentrations of cyanide ranging from m/27400 to m/274, the inhibitory effect of the cyanide and accelerative effects should *just balance one another* in practically every concentration between and including these limits! However, not being content with leaving this question without experimental test, I give below the results

TABLE 3

Showing the rate of oxygen consumption by Paramecium caudatum and its independence of the hydrogenion concentration in the medium. Paramecia used in this experiment were transferred to tap water and starved 18 hours previous to the experiment. Volume of bottles 136 cc. Temperature 19°C. Duration of test 36 hours. Range of pH of tap water to highest concentration of KOH in tap water was 8.2 to 9.5. The numbers in the table represent cubic centimeters of thiosulphate equivalent of oxygen

BOTTLE	CONTROLS			ANALYZED AT END OF 36 HOURS; 2 CC. PARAMECIA ADDED TO EACH BOTTLE						REMARKS
	Analyzed at once		Analyzed at end of 36 hours. 2 cc. paramecia added. No KOH	Cubic centimeters of m/10 KOH added						
	Blanks	2 cc. Paramecia added		0.2	0.4	0.6	0.8	1.0	2.0	
	cc. thio.	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	
1	4.60	3.38	2.75	2.58	2.70	2.90	2.60	2.70	2.52	All Paramecia living and normal at end of test
2	4.50	4.30	2.65	2.80	2.60	2.70	3.00	2.70	2.90	
3	4.52	4.40	2.77	2.78	2.80	2.70	2.50	2.70	2.70	
Average...	4.54	4.36	2.72	2.72	2.70	2.76	2.70	2.70	2.71	
Average cubic centimeters of thiosulphate equivalent of O_2 consumed.			*1.64*	*1.64*	*1.66*	*1.60*	*1.66*	*1.66*	*1.65*	

of one experiment designed to determine whether the hydrogen ion concentration of solutions represented by those of the cyanide and alkali solutions employed in table 2 above, and similar to those in the experiments on oxygen consumption (3), does have any effect upon the rate of respiration.

The striking uniformity of rate of oxygen consumption by Paramecium in solutions of KOH whose range of pH is from 8.2 to 9.5, is evident from the figures representing the average oxygen consumption by identical 2 cc. lots of Paramecium suspension. There is no trace of a stimulating or accelerative effect on the respiration.

TABLE 4

Showing that the rate of oxygen consumption in solutions of KNC is not changed by the addition of an equivalent amount of HCl to reduce the alkalinity. A dense washed suspension of animals was used. Temperature 19°C. Duration of test 17½ hours. Numbers in table represent cubic centimeters of thiosulphate equivalent of oxygen

BOTTLE	CONTROLS				ANALYZED AT END OF 17.5 HOURS 2 CC. PARAMECIA ADDED TO EACH BOTTLE					
	Analyzed at once			Analyzed at end of 17.5 hours 2 cc. Paramecium added	Cubic centimeters m/10 KNC added			Cubic centimeters of m/10 KNC + cubic centimeters of m/10 HCl added		
	Blanks	2 cc. Paramecium added	2 cc. Paramecium + 2 cc. m/10KNC + 2 cc. m/10HCl		0.4	1.0	2.0	0.4 + 0.4	1.0 + 1.0	2.0 + 2.0
	cc. thio.	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*
1	4.70	4.42	4.55	1.55	1.0	0.75	1.02†	1.10	0.83*	0.96†
2	4.68	4.25	4.35	1.60	1.0	0.95	.70†	0.35	0.55*	0.80†
3	4.75	4.40	4.45	1.35	0.6	0.55	1.20†	0.40	0.65*	1.25†
Average...	4.71	4.36	4.41	1.50	0.86	0.75	0.97	0.61	0.67	1.00
Average O_2 consumed cubic centimeter of thiosulphate				2.86	3.50	3.61	3.39	3.75	3.69	3.36
Average of all					3.50			3.60		

† = Less than 2 per cent of Paramecia dead.
* = Very few dead.

Will neutralization with an acid of KNC solutions unmask the supposed inhibitory effect of cyanide upon the rate of respiration in Paramecium? A different way to isolate the supposed accelerative effect of high alkalinity from the supposed inhibitory action on Paramecium due to cyanide as such, is to determine whether or not solutions of different concentrations of KNC which have been brought to practically the same hydrogen ion concentration by adding a proper amount of acid (HCl), have any effect on the rate of respiration.

Three concentrations of KNC were used; these were made up by adding 0.4 cc., 1.0 cc. and 2.0 cc. m/10 KNC respectively to each bottle in three sets of three bottles each. Then 2 cc. of Paramecium suspension were added to each bottle of 137 cc. volume. A second set of bottles was prepared in the same way except that in addition to the KNC, an equivalent quantity of m/10 HCl was added to reduce the alkalinity of the KNC solutions. A set of controls without cyanide or acid was also supplied in order to compare the rates of oxygen consumption in cyanide, and neutralized cyanide, with that of duplicate samples of Paramecia in tap water. The results are given in table 4. The total consumption in 17½ hours in KNC and neutralized KNC was about $\frac{1}{9}$ of the total quantity of oxygen in the bottle. There can therefore be no question whatever of the significance of the numerical values representing the average total oxygen consumption which is given in cubic centimeters of thiosulphate in table 4. The average oxygen consumption by the animals in tap water was equivalent to 2.86 cc. thiosulphate, the average of *all* the lots in KNC was 3.50 cc. and the average of *all* the lots in neutralized KNC was 3.60 cc. thiosulphate. No difference in the different concentrations of KNC is apparent. The solutions containing 2.0 cc. KNC and 2.0 cc. neutralized KNC were both about equally toxic as shown by the fact that some of the animals in both were dead at the time when the experiment was stopped. The death of animals and consequent decrease in the oxygen consumed is barely noticeable in the lower values of the average total oxygen consumed by the animals in the bottles to which 2.0 cc. of the KNC had been added.

The hydrogen ion concentrations in all the bottles which contained KNC and acid was very nearly the same as that of the tap water, as was shown by colorimetric tests. While the range of hydrogen ion concentration in the bottles containing 0.4 cc. 2.0 cc. KNC was 8.4 to 9.4. In this experiment there occurs an apparently distinct accelerative effect by the KNC on the rate of respiration. This acceleration amounts to about 25 per cent of the normal rate of respiration, but this accelerative effect is not due to the alkalinity of the solutions, as Child suggests. Further study of this phenomenon is necessary before any definite statements can be made as to the conditions for its occurrence and actual magnitude.

It will be clear from what has been presented thus far that the results from different experiments confirm one another, and in every detail confirm the results and conclusions of the writer's previous studies on the effect

of cyanide upon Paramecium. Furthermore all the suggested explanations by Child and Hyman of the difference in action of cyandie upon Paramecium and Planaria fail entirely to stand the test of experiment. The question still remains, why does KNC act differently upon Paramecium than upon Planaria? Some definite light will be thrown upon this question in a later paper.

A test of Child's explanation of the differences in survival time in KNC of fed and starved Paramecia and Planaria. It is a striking fact that fed Planaria, Didinia and Paramecia—and perhaps the same will be found for other animals—are more resistant to the toxic action of cyanide than starved animals of the same species. Similarly due to the action of food, the same animals after feeding have a higher rate of respiratory exchange than starved animals. The apparent discrepancy between these facts and Child's explanation of the relation of susceptibility to toxic agents and the rate of metabolism, or more specifically the rate of respiration, has been discussed in several papers by Child and his co-workers. It is maintained that in reality high susceptibility is correlated to high rate of respiration and low susceptibility to a low rate of oxidation. A full discussion of the question will be found in Allen (17). Child (4, p. 255 and footnote, p. 256) and Hyman (7, p. 398) have offered an explanation of the apparent discrepancy between theory and facts by making what appear to be two assumptions. First: That the toxic action of cyanide is primarily, if not entirely, upon the superficial structures of the animal, for example, in Planaria its action is assumed to be upon the body wall and in Paramecium the assumption is that the cyanide acts upon the ectoplasm. Second: It is assumed that when Paramecium or Planaria are fed, the increment of increase of respiration is localized largely if not entirely in the intestine of Planaria and the endoplasm of Paramecium, consequently the rates of respiration of the body wall and intestine in Planaria are more or less independent variables, a similar reasoning applies to endo- and ectoplasm in Paramecium. It is clear that since in the direct measurement of the respiratory exchange the results represent the algebraic sum of the rates of respiration in different parts of the cell or organism, it might conceivably be true that susceptibility tests with cyanide which according to the assumption acts only upon the body wall, might give different results from those obtained by direct measurement of the total oxygen consumption and yet not be out of harmony with Child's conception of susceptibility insofar as it may be related to rate of respiration.

This explanation may appear at first sight to have some merits, since it is probably certain that oxidations proceed at different rates in different parts of the same cell and since it is well known that different organs and tissues have different rates of respiration (19). However, there are two sets of facts which seem to speak fatally against this explanation of the difficulty.

First: Specific dynamic action of foods in higher organisms has been shown to be an accelerative effect upon the respiration of the body cells and not an effect residing in the alimentary tract, so that if we are permitted to reason by analogy from higher animals, we have certainly no *a priori* reason for believing that the effect of food on respiratory exchange in Planaria or Paramecium is localized in the intestine or endoplasm respectively.

Second: The most fatal objection is the fact that cyanide inhibits the oxidations in about equal percentage amounts of the normal rate, in both *starved and fed* Planaria. Now if, as we are told by Child and Hyman, the cyanide only or primarily acts upon the body wall and since food accelerates only or primarily the respiration in the intestine, then surely we should be able to note a very large and *distinct* difference in the degrees to which total respiration is inhibited in starved and fed Planaria when subjected to the same concentration of KNC, since according to Child and Hyman the action of cyanide is primarily on the body wall.

Tables 5 and 6 are two separate but similar experiments, the results from which show that the percentage inhibition in starved animals with 1 cc. m/10 KNC is 48 per cent and 47 per cent respectively, while the percentage inhibition by the same concentration of KNC in the corresponding fed lots of Planaria is 53 per cent and 63 per cent respectively. Evidently the KNC acts upon the respiration of internal organs as well as that of the body wall or else we must conclude that the specific dynamic action of food is on the body wall as well as internal organs. In short cyanide acts to a large extent, if not entirely upon the respiratory processes in the *same cells* whose respiratory processes are affected by the specific dynamic action of the food. The conclusion must therefore be that the interpretation of the difference in survival time in KNC solutions of fed and starved Planaria (and therefore also for Paramecium so far as Child's argument is concerned) on the basis of difference in rate of respiration in body wall and intestine is completely unwarranted. In fact, the percentage inhibition appears to be greater in fed than in starved animals which is directly opposite to what one would expect if the proposed explanation were correct.

TABLE 5

Comparison of the amounts of inhibition by KNC of the rate of oxygen consumption in fed and starved Planaria agilis. Animals starved 12 days before feeding beef liver to set B. Both starved and fed sets of animals were again starved 17 hours before the test. Duration of test 8½ hours. Volume of bottles 102 cc. Twenty animals in each bottle. The individual lots of 20 animals each were weighed just before feeding animals of set B. Numbers in the table are the actual number of cubic centimeters thiosulphate equivalent of oxygen consumed during the test. The weights of the different lots of animals were very closely the same and hence weights are not given in the table

BOTTLE	BLANKS. CONTROL = TOTAL O_2 AT BEGINNING	STARVED SET A		FED SET B		
		No KNC	1 cc. n/10 KNC added	No KNC	1 cc. n/10 KNC added	
	cc. thio.	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	
1	3.25	1.63	0.90	2.36	1.17	All animals living and normal at end of experiment
2	3.25	1.68	0.90	2.26	1.08	
3	3.25	1.72		2.26	1.00	
Average consumed during test		1.68	0.90	2.29	1.08	
Average consumed per gram during test		6.64	3.46	9.27	4.31	
Per cent inhibition by KNC			*48*		*53*	

TABLE 6

Comparison of the amounts of inhibition by KNC of the rate of oxygen consumption in fed and starved Planaria agilis. Animals starved 14 days before feeding beef liver to set B. Both starved and fed sets of animals were again starved 15 hours before the test. Duration of test 8 hours. Otherwise the procedure was exactly as given in table 5

BOTTLE	BLANKS. CONTROL = TOTAL O_2 AT BEGINNING	STARVED SET A		FED SET B	
		No KNC	1 cc. n/10 KNC added	No KNC	1 cc. n/10 KNC added
	cc. thio.	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*
1	3.20	1.52	0.77	2.16	0.84
2	3.15	1.44	0.88	2.24	0.84
3	3.20	1.48	0.84	2.16	
Average consumed during test		1.48	0.83	2.19	0.84
Average consumed per gram		7.87	4.12	11.34	4.20
Per cent inhibition by KNC			*47*		*63*

Further careful study and comparison of the effects of KNC on fed and starved animals may in all probability throw very important light upon the nature of the specific dynamic action of food.

CONCLUSION

It will be clear from the foregoing experiments which were designed to test briefly the validity of the criticisms and explanations offered by Child and Hyman, that not one of these criticisms or explanations has stood the test of experiment and careful examination. As a matter of fact a careful study of the data in the tables of the writer's previous papers should have obviated most if not all of the criticism in regard to the earlier experimental results. The writer has with some reluctance ventured into this reply to criticism since he has felt that in any situation where individual judgments differ in regard to the interpretation of experimental results, it is desirable to pay more attention to rigorous formulation of experiment than lengthy verbal discussion. From this standpoint the writer may be pardoned for this reply to criticism since he has attempted to answer by experiment rather than by explanations which are based on assumptions.

SUMMARY

1. Potassium cyanide even in concentrations which cause cytolysis do not decrease the rate of respiration in Paramecium caudatum. This confirms the writer's previous results. Paramecium therefore differs markedly from Planaria and most other cells and organisms whose respiration rate is decreased in the presence of KNC.

2. The criticisms offered by Child and Hyman of the experimental results and conclusions by the writer are shown to have no basis in fact.

3. The toxic action of KNC upon Paramecium is not due to the alkalinity of the cyanide solutions, but is due to the action of the cyanide as such.

4. The alkalinity of the solutions used in the experiments on Paramecium does not effect the rate of respiration, contrary to the suggestions offered by Child. Furthermore neutralized solutions of KNC are not different in their action from non-neutralized solutions of KNC. Whether or not KNC as such has a tendency to accelerate the oxygen consumption by Paramecium is an open question.

6. The assumption by Child that the rate of respiration in the body wall of Planaria is not primarily affected by feeding, and that KNC only or primarily affects the body wall and superficial structures, is not correct. For experiment shows that the percentage inhibition of the respiration in fed animals is just as great or even greater than the percentage inhibition in starved animals used as a control. In view of this fact similar assumptions which are made by Child and applied to endo and ectoplasm of Paramecium are unwarranted.

7. The explanation as to why Paramecium and Planaria differ in their behavior toward cyanide is to be sought in an entirely different direction from that suggested by Child and his co-workers. A following paper on the relation of oxygen concentration and its relation to the action of KNC will throw light on this question.

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GRADIENTS OF VITAL STAINING AND SUSCEPTIBILITY IN PLANARIA AND OTHER FORMS

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The investigations described in this series were undertaken in an attempt to reveal more facts as to the nature of the metabolic factors controlling individual organization and development, and to assign to these factors their proper relative values. Since recent work along this line rests largely upon a definite background of previous work, particularly that of Child, some of the conclusions of this writer may be summarily reviewed, with the caution that no statement or interpretation should be attributed to him without first consulting his own writings (1), (2), (3).

After extended experimentation and careful analysis of phenomena of regulation, growth and development in many organisms, Child put forth certain helpful generalizations as to the dynamic nature of the organism, which were applied convincingly to most varied and apparently independent groups of data. According to this view it is held that: metabolism is the basis of the phenomena of life, and an axiate "organic individual in its simplest terms" consists of a quantitative "metabolic gradient, or gradients in certain metabolic reactions, perhaps oxidations, with associated protoplasmic conditions," existing along the main axis and probably also in minor axes; the establishment of such a physiological gradient or gradients by interaction of environment and specific protoplasm is the first prerequisite to development and organization, and constitutes the basis of the functional and structural symmetry and polarity of the individual; through transmission of excitations the region of highest metabolic activity in the axial gradient exerts a dominating and integrating influence over subordinate levels with a lesser metabolic rate, such dominance or control being manifested by a correlating, coördinating, and generally unifying action in ontogeny, growth, regulation, behavior, etc.

Proofs of the existence of such a metabolic gradient and evidence of its nature may be found in the literature cited. These proofs and evidences have hitherto concerned themselves chiefly with differences along the axis in regulation capacity, in susceptibility, in output of CO_2, in consumption of O_2, and in electrical potential. Numerous other differences, often closely associated with metabolic activity, might well be sought for and studied in favorable forms, e.g., differences in heat production, in electrical conductivity, in H ion concentration, in water content, in permeability of membranes, in state of dispersal of colloids, etc. The rôle of each of these factors deserves individual attention, especially because of the wide applicability of the results in physiological gradients and in metabolism generally.

The writer believed that an attack of the problem might be made by a study of the action of electrolytes and dyes. Certain aspects of H ion action have been treated (4), and results with salts will be reported later. The object of the present paper is chiefly to state the experimental facts as observed regarding gradients of staining and susceptibility in several flatworms, protozoa, hydra, annelids, and the chick embryo with vital and other dyes; to analyze and interpret as far as possible these results in their bearing upon the concept of metabolic gradients as a further test of its validity and applicability; and, specifically, *to ascertain whether regions of high general susceptibility and rapid respiratory exchange behave in a characteristic manner in the staining process* as shown by the diffusion, segregation, flocculation, etc., of the dyes. The results are believed to contribute additional proof, with agents hitherto little employed, of the reality of the metabolic gradient in the forms used, and further evidence as to the nature of these gradients, particularly with regard to certain physico-chemical properties associated with high metabolic activity.

The experiments were performed for the most part at the University of Chicago in 1916–1917; the paper was then put in substantially its present form. Now newer observations and more recent literature are included. While the writer naturally assumes full responsibility for the results embodied in this work, he gratefully acknowledges his debt throughout to Dr. C. M. Child for many kindnesses and for the unfailing suggestiveness of his writings and criticisms.

GENERAL STATEMENT OF RESULTS

As might perhaps have been anticipated, there is a rather clean-cut difference between basic and acid dyes in their staining capacities

intra vitam, corresponding to the known differences in their physical and chemical properties. Basic dyes alone truly and definitely stain the tissues of the organisms under observation; though acid dyes sometimes penetrate and are even stored in granules, they do not in general become visible by fixed staining of protoplasm. Basic dyes of most varied chemical constitution and relationship were used but, while they differed very considerably in toxicity, in irritating action and in details of staining, the final staining pictures obtained with all were essentially similar. Most basic dyes, as toluidin blue, Victoria blue, crystal violet, methylene blue, janus green, etc., and even neutral red, are much more toxic than the acid dyes, as congo red, eosin, erythrosin, trypan blue, methyl orange, acid fuchsin, orange G, etc.

A given tissue or layer does not stain uniformly and simultaneously throughout the length of the specimen, even though at final saturation just before death the intensity of stain may become approximately equal everywhere. Regions of strongly marked susceptibility to such lethal agents as had been used and to acids, alkalies, and the dyes themselves, are regions where the basic dyes first became detectable in certain granules or globules of the cells. Thus in general a staining gradient is produced indicating directly the metabolic gradient. A gradation of penetration was found with every major gradation of susceptibility.

Depth of coloration increases rapidly as the death point is neared, but preliminary to the actual onset of disintegration there occurs in most species with most basic dyes a sudden loss of both natural pigment materials and stained particles, leaving the most susceptible parts strikingly decolorized.

In causing a selective disintegration methylene blue and some other dyes proved to be favorable agents for demonstrating not only the chief longitudinal axis in flatworms but also in many cases the minor axes as well. But these axes were not always to be distinguished by differences in staining, nor is there noticable difference in rate of staining of young and old individuals.

EXPERIMENTAL

Methods. Dye samples were obtained from as varied sources as possible (Grubler's, also Bausch and Lomb's and Kahlbaum's). Stock solutions were made up in well or tap—rarely in distilled—water, and were not made free from the salt impurities with which they were dispensed.

In vital staining it is highly important that the experimental animals be healthy and that they be kept under almost continuous observation to

watch the progress of the staining since very erroneous notions may be obtained by examining the specimen at the end of the process when tissues have been loaded to saturation. The staining should be witnessed as a process rather than observed in the finished state. Animals were usually brought under observation in clear water and closely examined from time to time with eye, lens, dissecting or compound microscope, and were often flattened under a cover slip with or without support, or teased.

The salient features of *the susceptibility method* have been many times described. According to the concentration of chemical agents or the intensities of physical conditions used there are two general modes of studying relative susceptibility, the direct and the indirect, both of which seem to have a definite and characteristic relation to the metabolic rate. With the *direct* method such concentrations or intensities are used as are lethal within a few hours; in this case individuals or parts with highest metabolic rate are most susceptible, and the susceptibility gradient follows the metabolic gradient. With the *indirect* method such lower concentrations or intensities are used that some acclimation occurs and death takes place only after many hours or days; in this case acclimation is most rapid and complete in individuals or parts with highest metabolic rate and length of life varies directly with rate of reaction.

As *criteria of* relative *susceptibility* use was made of loss of motility or response to stimulation, possibility of recovery, etc. For most lower invertebrates disintegration is a satisfactory index, and appropriate for fairly exact and quantitative readings. Death is manifested by a loss of continuity of surface contour, swelling, and loss of constituents, by the visible separation of tissue fragments and cells from the main mass, and by change to acid phase of some dye indicators (e.g., neutral red). With the usual slight disturbances in the container tissue fragments continue to scatter out into the medium until little or nothing remains *in situ* of the dead parts. A disintegrating organism thus imparts a turbidity and a tinge of color to clear water. Impending disintegration is often indicated by cloudy swelling, opacity, immobility and loss of local responses. Accompanying color changes consist of loss of more or less natural pigment or previously stored stain. Criteria of equivalent staining rates are those of direct observation and readings on the time required for first visible coloration of a given part.

Factors modifying the effect of dyes: Concentration. Figure 1, summarizing averaged data collected from many protocols, shows the

fairly direct relation existing between concentration and time of first staining, staining at all levels, and first disintegration of *Planaria dorotocephala;* vital staining is roughly a function of time and concentration. The interval elapsing between a given intensity of staining and the initial disintegration widens with increasing dilution, until the

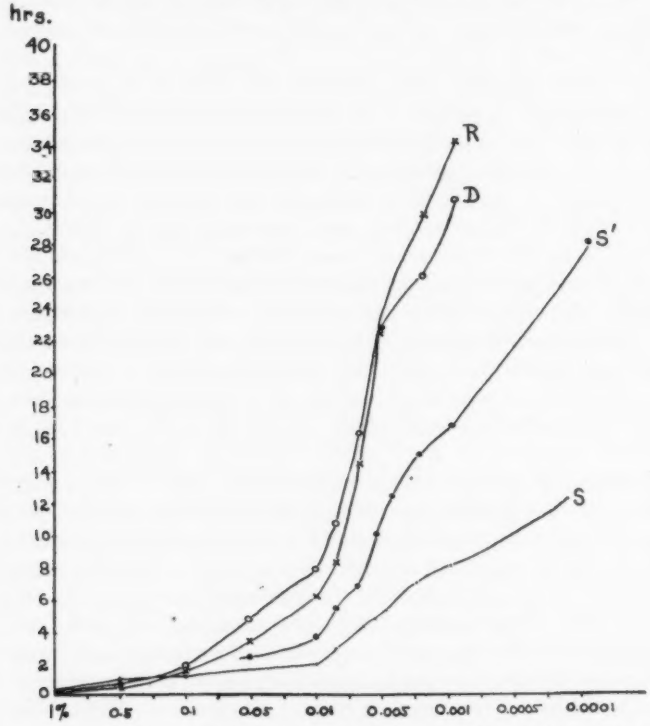

Fig. 1. Times of first visible staining, *S*, of first visible staining at all levels, *S'*, of first disintegration, *D*, and of longest possible exposure with complete or partial recovery, *R*, of *Planaria dorotocephala* of about 15–18 mm. length in different per cent concentrations of methylene blue at room temperature.

animals, though ultimately well stained, disintegrate less and less completely with greater individual variations, and finally near 0.0001 per cent will live on stained for an indefinite period either wholly intact or after recovery with loss of head and other most susceptible parts. In less toxic dyes like neutral red or even dilute methylene blue the worms

survive for months, carrying the dye even past fission and regeneration crises. In 0.1 per cent methylene blue loss of head substance occurs before stain has become at all visible in caudal regions, and in slightly higher concentrations toxic effects may be produced without appreciable visible staining, for recovery is impossible when staining begins. In short the curves of staining, S, S', do not run parallel with that of recovery, R, or that of initial disintegration, D. With a given intensity of stain prognosis for recovery is least favorable from strong and practically certain from weak solutions. Disintegration and failure to recover apparently depend less upon actual staining than upon presence of excess stain in the medium.

So far in this work the reversed order of susceptibility (indirect) has not been met with even in most dilute solution. But mere traces of methylene blue are said to have an accelerating effect upon growth of yeast.

Age of the organism is doubtless a factor in determining dye action. Ten large and ten small planarians were immersed together in 0.1 per cent methylene blue and examined at half-hour intervals and records made of the progress of their disintegration. A graph of the results shows a little difference in the resistance of the two sets of animals, the younger ones being perhaps slightly more susceptible than the larger. Similar results are obtained with acids (4).

Temperature. At 14°C. staining in 0.02 per cent m.b. is strikingly delayed as compared with that under the same conditions at 23°C. The protective effect of cold on organisms in the dye is so marked and the rates of staining and disintegration are so similarly modified that the clue might be followed further for evidence on the exact value of the temperature coefficients for intake of dye and for disintegration—particularly since amount of adsorption has a negative temperature coefficient (5). It would seem that adsorption is soon followed by chemical combination.

Hydrogen ion concentration. The reaction of a dye solution is of prime importance. Staining in well or lake water of pH = 7.5 may be considered rapid; if this water be made more alkaline by the addition of NaOH rate and depth of staining increases with rise of pH. At the same time in the more alkaline media differences in sites of staining also appear in that certain irregular or stellate bodies with nucleus-like centers stain conspicuously on the ventral surface, and in that blue granules are detectable in the posterior zoöid region of *P. dorotocephala* very shortly after similar ones are visible anteriorly and before any are

to be seen at intermediate levels. As OH′ is reduced by addition of HCl the staining becomes more and more limited to the auricles and tip of the head (pH = 6.4), and finally at pH = 4.8 stained tissue cannot be found anywhere even after hours of exposure, when the acid itself kills. Probably for this reason animals stained very poorly in distilled water solutions of the dyes, for its reaction was about pH = 6.0. As a rule this species dies from the toxic effects of the distilled water before more than the few most sensitive parts of the head have been stained. In fact it is extremely difficult to get any basic stain at all into some specimens of oligochetes and protozoa when taken from an old, very acid culture. Sufficiently acid media apparently reversibly destain some vitally stained protoplasms to a certain extent; in many cases m.b. passes through a green or a more or less decolorized state. How much all these results are due to influence of the H ion concentration on dissociation and rate of diffusion of the dyes and how much to the alteration of membranes and deeper tissues is yet undetermined.

Susceptibility of all parts to a basic dye is usually increased by a definite alkaline reaction in the medium. In planaria the disintegration of the head is followed at once by the disintegration of the posterior zoöid region—an order which is not often followed if the reaction be acid. Alkalies seem to sensitize certain parts of the organism so that non-lethal concentrations of either the alkali or the basic dye combine to become lethal. It has been reported that alkalies increase direct susceptibility and acids indirect susceptibility (1), (4). But this fact should not be confused with another, namely, that an *inner* acid reaction probably increases suceptibility to basic dyes (6); both of these facts are consistently interpreted in the discussion.

Neutral salts markedly retard or actually prevent staining of all parts of planarians with m.b and other basic dyes. In this, $CaCl_2$ is more effective than NaCl. It is surely significant, however, that salts and hydrogen ion facilitate acid dye action.

Data in detail. The bulk of the data deals with the effect of m.b. on diverse species, and the results obtained are described for this dye with each form, frequent notes being added for other basic and a few acid dyes, when peculiarities or divergences in action were observed.

Planaria dorotocephala. This flatworm was singled out for particular study with the dyes because it was readily available and especially because it had been extensively used in the same laboratory for similar studies, and many data were already at hand of much value for comparison. Previous work with this had shown that individuals over

5 mm. are composed of a large anterior zoöid and of one or more smaller posterior zoöids not morphologically differentiated but clearly distinguishable physiologically (1). With these in mind attention was devoted chiefly to the relative speed and intensity of staining of different levels along the axis and to the time of disintegration of these different levels.

Staining gradient. Immersed in a solution of basic dye, planarians do not stain uniformly and simultaneously even throughout their surface area. Certain points of election are from the first visible. In general

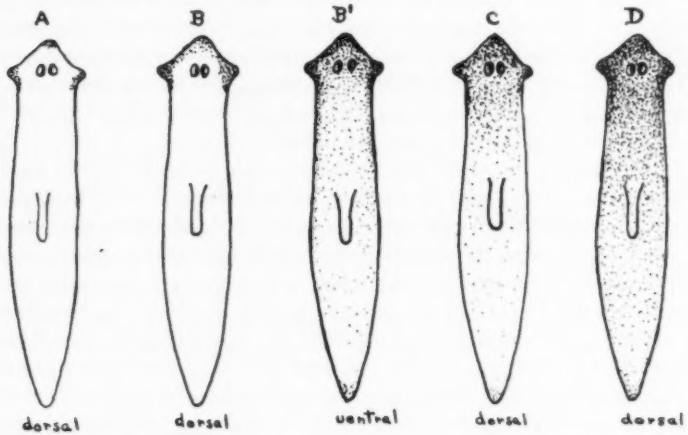

Fig. 2. Stages of staining of *P. dorotocephala* with methyene blue (and many other basic dyes) to show sites and relative intensity of coloration: *A*, stain in sensory lobes chiefly; *B*, spreading along margins of the head, and *B'*, same stage in ventral view, stain extending much farther than in *B*; *C*, and *D*, continued deep staining in more posterior levels. In alkaline media or with very young worms the stain shows early also in the posterior tip.

the first staining occurs within a few minutes or hours in certain parts of the epidermis. This order of such staining (fig. 2) is, practically without exception, first the lateral auricles, *A*, then the tip and later the ventral surface and margins of the head, *B*, *C*, continuing posteriorly thence until the whole animal excepting the proboscis is distinctly bluish, *D*, and finally quite blue-black externally. By use of diluter solutions and removal of specimens to clear water from time to time at appropriate intervals a series is obtained showing stained areas extending progressively backward and intensifying. At an early stage the animal is

quite distinctly "cyanocephalous" and is much given to moving the head in constant exploratory movement, or holding it stiffly erect. The ventral surface of the head takes on and retains a deeper coloration than the caudal parts and the dorsal surface, this difference persisting through further staining until shortly before cytolysis begins. In concentrated solutions the staining becomes more equalized throughout, and the initial differences are less evident. Curiously the proboscis remains strikingly uncolored even to the last in many dyes unless treated in a special manner to induce staining. In later stages of deep staining the anterior end is rendered more and more inactive, flaccid and unresponsive, and the part previously held erect drops under the ventral surface and the remaining parts roll or coil dorsally at ends and at the margins, in a characteristic fashion. If stimulated the animals may yet unroll and glide stiffly with the posterior cilia or muscles, but the uplifted anterior end is not touched to the substratum.

Teasing and close examination show the dye to be located in certain stained droplets and granules as well as to a less extent in the ground substance, first of the superficial cells and later in the deep-lying tissue. As a rule the stained constituents prove to be chiefly globules of an ever increasing size and number, occurring singly or several together inside larger globules of a bluish liquid. In m.b. made strongly alkaline the stain appears to pick out and color a number of irregular cells with central nuclei (?) situated on the ventral surface; no attempt was made to localize this stain by study of prepared sections.

Repeated tests made in various ways by exposing to H_2O_2 planarians stained only cephalically gave no indication of there being any colorless leucobase present but invisible in unstained regions, where it might conceivably be reduced. In fact, as Ehrlich found for m.b. in nervous tissue (7), the expectation would be that regions of high oxidative metabolism would reduce dye compounds to a leucoform more rapidly than the less active parts here left unstained.

Injured loci, wherever situated, take up basic stains considerably in advance of any uninjured parts: fission planes, either freshly or recently exposed or after the ends have contracted down and begun healing and reconstitution, exhibit a similar precocious coloration. There is no observational evidence for believing that simple exposure of or removal of a membrance from interior substances will promote immediate staining; the increased staining is such as would be expected to proceed from the stimulation of injury or the higher metabolism of contracting ends. It is interesting also to note that regenerating heads,

however translucent and apparently devoid of "density" and of differentiated structural material, yet stain easily and relatively deeply.

Previous killing by slow heating or by alcohol allows stains to flood in rapidly at all levels. Only in life was the staining gradient with basic dyes obtained.

Disintegration gradient. As bilaterally symmetric animals, flatworms possess three axes: the chief, antero-posterior axis, a ventro-dorsal one, and a medio-lateral one in the horizontal plane. Each of these axes should theoretically be represented by a gradient in metabolic or protoplasmic condition, such that a region of highest rate might be distinguished by its more marked susceptibility from other regions of lower rate.

As has been stated, there are low concentrations of stains which will stain without producing lethal effects anywhere. Once this minimal concentration is passed it is only a question of time when disintegration will set in. After the necessary toxic effect has been produced, the epidermal cells of the anterior end along the auricles and tip and ventral surface of the margins of the head assume a swollen and edematous aspect, loosen up from each other, lose their coherence and their original structural orientation, and scatter in small shreds, clumps and spherical masses, usually as small as the globules or granules composing the protoplasm. A disintegrating area decolorizes somewhat by extrusion of the stained constituents, so that the disintegrating portion is often sharply contrasted with the intact blue portion, as a loose, white, felt-like, downy tuft.

The order of disintegration of the epidermis and body wall (fig. 3) is most significant, since it affords an excellent readily visible demonstration of all of the three gradients believed to be present in the outer layers of the triaxiate organism. *a.* The zone of decoloration and of disintegration begins invariably on the auricles and tip and margins of the head, and proceeds slowly caudad. The disintegration belt is constantly shortened in front by detachment and loss of granules and droplets, and lengthened behind by the incorporation of more sound tissue into the disintegrating zone, which finally reaches the extreme posterior end. The belt immediately behind the disintegrating level is strongly contracted as in the sphincter-like closing of any injured part. Meanwhile the attitude of the head, margin and body is as described above. Parts left intact usually will exhibit some movements upon stimulation; only the more posterior levels retain power of adhering to the substratum. For a few minutes preceding initial

disintegration the individual passes through a stage of rhythmic movements; the wave proceeds postero-anteriorly—the posterior tip extends to its maximum caudally and then widens and shortens from the caudal tip forward, as if in attempting backward movement of an avoiding reaction. In moderate concentrations these rhythms may reverse in direction, several times alternating from posterior-anterior to anterior-posterior. *b*. The ventro-dorsal axis is also clearly indicated. Whitening and dissolution of tissue usually extend caudad more rapidly on the

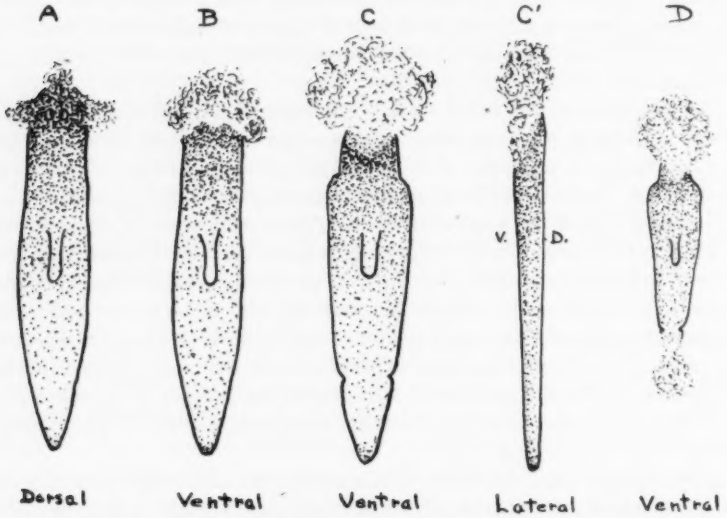

Fig. 3. Certain stages of disintegration of *P. dorotocephala* in methylene blue, showing correspondence with stages of staining of figure 2; and also the frequent precedence of ventral over dorsal disintegration, *C'*, and of median over lateral disintegration, *C*; *D*, and *D* the posterior disintegration of younger worms, or of any worm in a definitely alkaline medium of the dye.

ventral surface than on the dorsal (fig. 3, *C*). This more rapid advance of disintegration ventrally is obvious from the time the first tissue is lost under the head and is still noticeable posteriorly; in fact in many cases the ventral surface has entirely disintegrated when the dorsal parts of the posterior end are still intact in the form of a carapace-like shield of tissue, which is the last to display irritability and to disappear. *c*. Even the median-lateral axis is often demonstrable. Disintegration of the ventral surface does not usually advance back-

ward on an even transverse front. Often, and especially in high H ion concentration, disintegration of the epithelium is most rapid in the midline, so that the decolorized and cytolyzed area pushes back in a wedge shape and the living tissue is cut out to show a V-shaped front with two lateral arms projecting forward. In dorsal view the picture is more variable, and toward the posterior end the line of disintegration becomes more transverse.

In younger animals the posterior tip commonly disintegrates soon after the head. In more alkaline media all animals disintegrate thus (fig. 3, *D*). In no case was there any early loss of tissue at the anterior end of the second zoöid with basic dyes. A recent fission plane shows early disintegration corresponding to its early deep staining.

With dilute cyanide and various anesthetics Child obtained first disintegration along the margins, at the posterior tip, and dorsally at the anterior ends of intermediate zoöids. These results could not be duplicated with any basic dye or with acids, but were approached closely by use of alkalies and some acid dyes, as alizarin blue S (fig. 4).

Fig. 4. An early stage of the disintegration of *P. dorotocephala* in alizarin blue S, and acid dye. Large specimens commonly show disintegration in the order *1*, *2*, *3*, *3'*, or *1*, *2*, *3'*, *3*.

Other basic dyes differ from methylene blue chiefly in the degree of their irritating and toxic effects and in minor details of their staining. Naturally those with colors differing widely from the natural yellow brown pigment are most favorable for study of penetration of the dye, but all lend themselves to use as agents in susceptibility work—neutral red, crystal violet, victoria blue, magenta red, janus green, toluidin blue. The last two perhaps best show early staining of the posterior zoöid region.

Acid dyes—eosin, erythrosin, trypan blue, methyl blue, water blue, berlin blue, acid fuchsin, congo red, and many others—were tried in neutral solution but none became visible within the living animal even after hours or days. Only a few were toxic enough to kill. After death the dyes passed in but were easily washed out again. The action of alizarin blue S has been mentioned above. In more acid media the acid dyes are more effective, but here the acid effects seem to be predominant.

Planaria velata. In all essential respects this species resembles the above. Basic dyes penetrate and become visible within the proto-

plasm first in the truncate end and anterior margins of the head, and only later are to be seen at more posterior levels. *P. maculata* is even more similar to *dorotocephala*.

Phaenocora agassizi. This small white transparent rhabdocoel with large cilia was first met with and collected in abundance preying in a thriving ameba culture. On account of its small size (4–5 mm.) all observations were made under the microscope. This form is of some interest, for an individual is composed of a single zoöid and shows a simple steep main gradient. This gradient may be demonstrated with basic dyes in several distinct ways:

1. The staining gradient. Placed in 0.01 per cent m.b. the animal takes up the stain in a clearly differential fashion along the chief axis. A clear hyaline apparently structureless layer over the entire surface

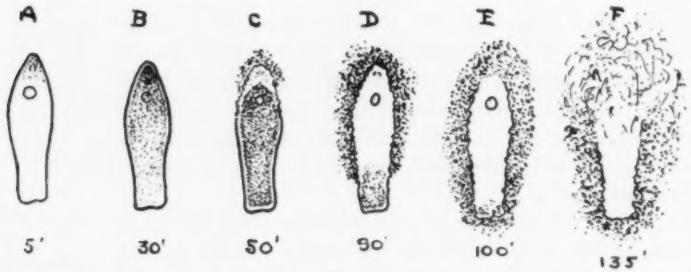

Fig. 5. The anterior-posterior gradient of staining *A*, *B*, *C*, of decolorization *C*, *D*, *E*, and of disintegration *E*, *F*, of *Phaenacora agassizi* in 1 per cent methylene blue.

remains relatively unstained. Almost at once the underlying tissues begin to stain around the sensitive point and edges of the reddish pigmented anterior end. It should be noted that this part lies well in front of the pharynx opening, contains no portion of the alimentary canal, and that no stain has yet become visible in the pharynx itself. The dye may be seen penetrating farther and farther caudad until in about 30 minutes its presence is indicated at the posterior truncate end. At this time the color differential is well marked; the pointed anterior end exhibits the first and the most abundant large blue granules which are progressively fewer posteriorly. Other concentrations give the same order of staining. The intensity of coloration continues to increase but the difference antero-posteriorly is never lessened until death changes are evident (fig. 5).

2. The gradient of extrusion of the stain. In about 40 to 50 minutes after exposure to 0.01 per cent m.b. the uncolored superficial layer in front of the pharynx assumes a somewhat bubbly outline as the cuticle is raised in small blebs. The edema or swelling in this zone doubtless implies changes in permeability and more or less local injury preluding death of the part, for closely following upon these alterations of state of membranes and tissues there ensues a conspicuous expulsion or escape of bluish granules and spherical clumps of cell material leaving the region without stain or pigment but surrounded by a blue halo or corona. The zone of extrusion slowly progresses backward and reaches the posterior end 45 minutes or an hour later.

3. The disintegration gradient. Immediately following the loss of colored particles a dissolution of structure sets in, manifested by further aggregation, clumping, swelling and lack of cohesion, fading away of the limiting epidermis and ultimate disorganization into semi-fluid transparent droplets or rounded granular masses. As superficial structures disappear and dissolve internal parts swell and push out. By the time colored particles are first thrown out from the intact posterior end, the anterior end is already disintegrating. At $2\frac{1}{4}$ hours disintegration has completely obliterated anterior structure and has been carried well toward the caudal end, the sound tissues being demarcated always by a sharp and well-defined boundary. Apparently in all cases the decoloration as well as the breakdown of tissue, once begun, is more rapid and simultaneous posteriorly, as if the gradient of susceptibility were more level and uniform there, though steeper anteriorly. That disintegration follows close upon death is indicated by the continuance of the ciliary stroke to the moment of disintegration when the beat becomes feebler and crawling and finally comes to a full stop.

Dalyellia (*Vortex*) *viridis*, also stains expels stained particles, and disintegrates in a definite anterior-posterior order.

Bothrioplana alacris? A small white rhabdocoel, evidently with triclad affinities, was collected from a temporary spring pond, but the sudden failure of the material left its identity uncertain. In 0.002 per cent m.b. staining begins definitely anteriorly, attacking the tip and especially the ciliated pits on the margin of the head. In 20 minutes the gradient is marked; at 30 minutes posterior parts are not yet stained, except sometimes at the extreme caudal end. In 40 minutes disintegration starts in ciliated pits and margins of the head and extends backward at such a pace that the anterior half of the

animal is removed in 55 minutes and the whole body scattered in 1½ hours.

Stenostomum leucops. As collected in April and May in indoor cultures this form consisted largely of chains of from 2 to 5 zoöids, but there were individuals in nearly all stages of fission and regeneration. Since a relatively high degree of differentiation (ganglia, ciliated pits, pharynx, etc.) is attained before zoöids separate from the chain, *Stenostomum* should be contrasted with planarians in which the posterior part is removed in a much less developed state.

A posterior zoöid of a 2-zoöid animal recently divided shows, if any, only a weakly developed secondary zoöid so that the gradient is simple and straight. The stain becomes visible first in the ciliated pits, then extends superficially to other more posterior levels. It finally seems to strike deeper and reach the ganglia underlying the pits, the other nerve structures, and sense organs around the mouth. Disintegration follows in 1 to 2 hours in 0.02 per cent m.b., usually before much stain is detectable posteriorly. Around the pits and over the main nerve masses the tissue swells even to rupturing, the protoplasmic masses taking deep stain when thus exposed. Hence in this case the disintegrating part is bluish. The ventral (oral) surface may disintegrate somewhat more rapidly than the dorsal surface.

An anterior zoöid recently detached from its posterior one stains and disintegrates at both anterior and posterior ends; the posterior end being both stimulated and exposed at the point of separation. Sometimes the fission end is lost well before the anterior end. In practically every instance disintegration of the anterior end follows the rule of midventral precedence over lateral and dorsal parts. In very acid media only the pharynx wall stains—in a sort of a network.

In an intact 2-zoöid animal the anterior end stains and disintegrates first; the new-forming anterior end of the second zoöid follows next in order, more rapidly if well formed, only after a time if not manifestly differentiated. A region where a fission septum is forming or where fission is taking place stains not only behind but also, and fully as much, in front of the septal plane. This region is doubtless subject to marked stimulation attendant upon the stresses and strains of the more or less violent separation which the dyes tend to induce. Individuals with more than 2 zoöids also tend to break up into discrete zoöids, but when the animal remains compound the zoöids stain as independently as in the 2-zoöid specimens.

Paramecium caudatum. Among protozoa *Paramecium* and *Dileptus* were chosen on account of their commonness in infusions, their elongate and axiate form, their comparatively exposed and uniformly ciliated surface, and the position of the oral aperture far back from the anterior end.

Most coarser stains, both basic and acid, are taken in and segregated in or near the food vacuoles of *Paramecium*, but m.b. and many other dyes of high visibility and marked color contrast also enter and stain somewhat diffusely even the less granular parts of the cell. With these agents, at 0.001 per cent or more, it is seen that individuals from actively dividing cultures show a distinct deep staining first in the extreme anterior end (fig. 6), soon concentrating below the ectoplasm in the outer endoplasm, in which the color spreads gradually backward; meanwhile the food vacuoles store much dye and collect posteriorly. In any effective concentration of the dyes the animals commonly

Fig. 6. Order of staining and disintegration of Paremecium in methylene blue.

reverse the direction of their swimming, or alternate in vigorous forward and backward movements, until they become sluggish and finally come to rest when they are heavily stained (except for the nucleus).

Shortly there occur changes in the gross appearance of the cell—the surface contour becomes more spherical, and the cuticle with its patterned markings, the cilia, etc., is lifted off the underlying parts and often ruptured, as if by inner swelling and the accumulation of a vacuole-like blister of fluid in some portion of the anterior end (not usually over the contractile vacuole). The fluid appears to force more and more of the more solid central contents posteriorly until the anterior portion of the cell has been practically emptied except of liquid while the caudal end is dense and crowded. The ciliary strokes cease soon after the outer layer is raised up, and always in the antero-posterior order, with many minutes intervening between their cessation in front and behind. It should be noted that only late in this process does the nucleus become deeply stained—a certain sign of lethal exposure.

This description applies quite generally for other basic dyes and for other typical ciliates, as *Stylonychia*, etc. Budgett (8) described antero-posterior dissolution of *Stylonychia* as a result of lack of oxygen, addition of KNC, pilocarpine, etc., and Child reported a somewhat modified gradient in several ciliates with KNC.

Dileptus gigas. This very large and elongate ciliate also reverses its direction of progression in the irritating dye solutions, until depression and paralysis ensue. In low concentration some basic dye is ingested and stored in the vacuoles, but in greater concentration little is thus taken in, and the animal gradually shortens and rounds out as it

Fig. 7. The gradient of intravital staining and of disintegration of *Dileptus gigas* in methylene blue and many other basic dyes.

becomes quiescent. The stain enters first at or near the tip of the proboscis, especially along the row of large ventral cilia which extend back toward the mouth (fig. 7). Thence it continues to become visible further back in the middle regions and finally at the caudal tip itself. Disintegration occurs either slowly, or sometimes suddenly, with a loss of substance of the proboscis tip and base, of the oral region, and so on; or in some cases quite differently by a series of ruptures along the dorsal side opposite the many contractile vacuoles.

Hydra oligactis. Hydra is also instructive, in providing a case of a radially symmetrical animal where secondary budding may be followed in all stages (fig. 8).

A young hydra without buds, placed in a Syracuse dish, allowed to come to rest and attach for a time, and then covered carefully with m.b. (e.g., about 0.002 per cent), shows blue granules first in the ectoderm of the tips of the tentacles, on the mound of the hypostome, and on the body below the bases of the tentacles. The tentacles load up rapidly with stain, especially at their ends which soon surpass all other regions in their intense blue coloration. Dye meanwhile is detectable more and more basally upon the column and in a half-hour or more may be found everywhere in the ectoderm, excepting sometimes in portions of the base of the stalk. Nematocysts are commonly discharged instantaneously by strongly irritant dyes, and may be seen heavily colored throughout, either attached *in situ* or thrown out into the medium. They are practically always extruded first from the

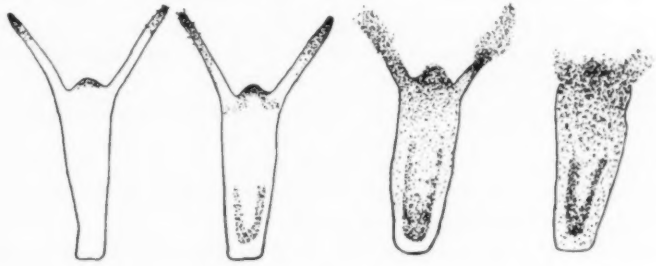

Fig. 8. Order of intravital staining and early disintegration of *Hydra oligactis* in many basic dyes.

distal parts of the tentacles, and then more synchronously elsewhere. (Incidentally, this action of irritant basic dyes provides an excellent method for demonstration and study of the kinds, numbers and distribution of the stinging cells, especially of the smaller kinds shown only indifferently well by acetic acid and methyl green.) When disintegration occurs it also begins distally in the tentacles, which are slowly removed bit by bit down to their bases, after which the hypostome and adjoining oral parts are similarly broken down. Basally there is less order and regularity in the disorganization. While the stain was entering the ectoderm it was also accumulating in the gastrovascular cavity in an irregular fashion, chiefly at the bottom of the cavity, and somewhat orally reaching out into the bases of the tentacles. It is this fact, that the dye is taken in by the large mouth and made available to the endoderm, which helps to complicate so much

the later stages of disintegration in larger hydras. But even this cannot conceal the essential fact that in hydra a gradient exists from tip to base in the tentacles, oral to aboral in the column as has been shown by Drzewina and Bohn (9) with lack of oxygen, heat and chemicals, and by Child and Hyman (10) with KNC and dyes.

If a well-formed bud is present, it takes the stain and disintegrates in about the same order as the young specimen. With regard to the time of first staining of the bud and the parent there is fair constancy of behavior; usually the parent tentacles stain earliest, accompanied or followed soon by bud tentacles, and then by the body of parent and bud. The rounded or cylindrical elevation where a new bud is forming, even though it be but a proliferating rudiment, exhibits a considerable capacity for early local staining. A bud eminence thus stains prior to the adjacent parent body. Disintegration of tentacles of a large bud and of the parent occur at about the same time; a small bud without tentacles is disorganized after the parent tentacles but before the parent body of the adjoining level is attacked.

Neutral red gave evidence of a similar gradient: from tip to base in a tentacle, and from hypostome downward on the column. To congo red and phenol red, which can hardly be said to stain, and even to hydrogen peroxide, hydra displays a like differential susceptibility for it succumbs gradientwise in these agents.

Hydra differs from most animals used in that it will take up and concentrate a basic stain from solutions so dilute as to appear clear.

Among the Annelids a number of common fresh water species were used. For purposes of comparison and confirmation data on these forms has fortunately often been available from the work of Hyman (11), who gives an interpretive analysis of the process of regeneration and demonstrates and describes the gradients of susceptibility to KNC in many oligochetes. It will be obvious that, except in minor respects, dyes show the same gradients.

Aelosoma hemprichii. This small form was collected readily from mixed protozoan cultures. The conspicuous structures are: reddish oil globules imbedded in the integument, and, apically, the flat rounded sensory prostomium, a ciliated pharynx, and cerebral ganglia just anterior to the pharynx.

Both staining and the ensuing disintegration proceed down a gradient of a primary sort (fig. 9). In an intact animal without fission planes the stain (e.g., 0.005-0.01 per cent m.b. for 1 to 4 hours) shows first in the ciliated pits and thickened sensory epithelium of the rounded ventral

surface of the prostomium and in the oral epithelium, but is soon visible also in more dorsal areas above the mouth between the pits and over and in the two ganglia lying superficially in full contact with the epidermis. From the deeply colored anterior end the staining area (and disintegration later) is carried backward at first slowly behind the mouth and then in more rapid sequence through the more posterior levels. In this progress stain does not enter segment by segment but in continuous gradation. The oil globules appear to stain about equally rapidly at all levels. The ciliated pharynx and the oral parts of the intestine may draw in and accumulate quantities of the dye, and produce a local area

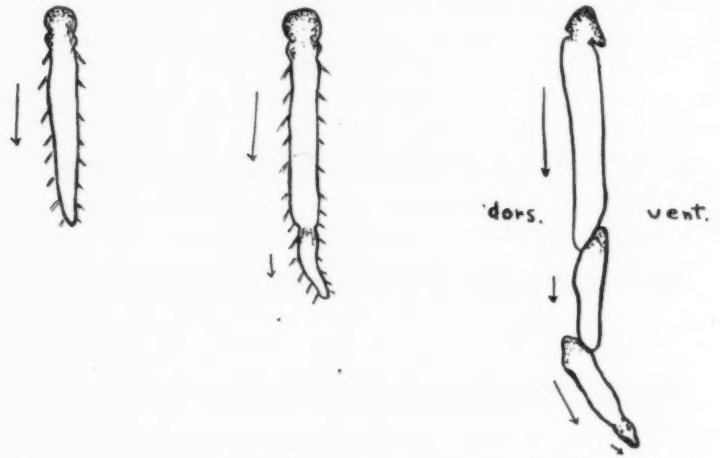

Fig. 9. *Aelosoma hemprichii*, showing order of staining of different specimens, composed of one, two or more zoöids.

of deep staining disturbing the simple gradient, but the first parts actually to stain lie wholly in front of the alimentary canal and out of communication with it. The posterior end stains early if a posterior zoöid has recently been removed.

If an animal possesses a marked fission plane, the stain enters in a definite ring of epidermis at or near each anterior end and concentrates, especially on the dorsal surface, behind the plane. The further course and times of staining are similar in both zoöids, which are ordinarily separated by the disintegration of the anterior end of the second.

Dero limosa. The important structures and their order of staining are shown in figure 10. In 0.01 per cent m.b. a sound specimen without

zoöids stains quickly in cutaneous portions, particularly anteriorly in the prostomium and ventral sensory buccal areas. An especially active ciliated gill region at the anal end composed of gills in a respiratory pit colors about as soon. Staining progresses slowly back from the head and more rapidly from the anal end forward. In this latter course it moves by segments or small blocks of segments, which stain first near the septa where, intersegmentally, a sphincter-like contraction takes place and forms a chain of blue bead-like rings, such a chain lengthening by additions from in front. In each segment the deepest mass of dye accumulates in ventral patches apparently corresponding in position to the segmental ganglia. The advance cephalad is soon met by

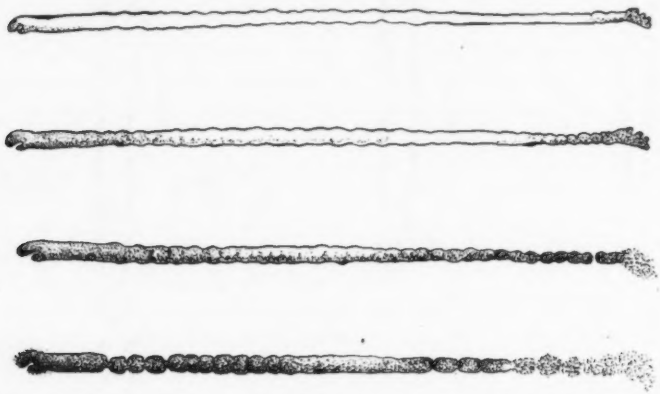

Fig. 10. Progress of staining and disintegration in *Dero limosa*, resembling generally that for most higher Oligochetes.

anterior staining moving caudad. In this and the higher annelids the cuticle seems to interfere with the penetration and fixation of the dyes, for in all staining is uneven at any given level and may wash out for some time after it has passed through the outer covering.

When two zoöids are present, the anterior stains the more darkly at first and in the antero-posterior order. A posterior zoöid stains less deeply, at first postero-anteriorly and later also from its anterior end back. As differentiation of cephalic structures proceeds in this zoöid these stain more and more quickly, finally equalling or even anticipating the anterior end of the first zoöid.

The first losses by disintegration are from the gill region and several of the posterior segments. Then follow the prostomium and the next

succeeding anterior segments. By the time the head goes the last 10 or 12 segments have been cut off one by one or in small groups. Intermediate portions may recover partially after losses from either end.

Lumbriculus inversitans. In 0.001 per cent m.b. epidermal structures stain chiefly at the anterior end and the caudal tip. The head stains quite uniformly along its length; it lacks septal divisions. Dye is soon visible more or less irregularly in intermediate regions, and edematous swelling occurs at corresponding points.

A part becomes pale blue on disintegration. The caudal third or more of large worms, beginning posteriorly, has formed a bead-like series of blue segments, as in *Dero*, by the time the head shows first signs of actual breakdown. The wave of further disintegration spreads backward from the head and forward from the anal end. Segment by segment the stain floods into the tissues and then is partly lost again in the subsequent dissolution process. Smaller and larger worms begin disintegration about simultaneously, but smaller ones may complete it sooner.

Tubifex tubifex and *Limnodrilus claparedianus.* Cutaneous parts, bristles, as well as the whole superficial nerve plexus take on stain early. In small, 1 inch specimens the anterior end colors first. In larger ones before the head segments stain perceptibly posterior ones have long since colored deeply and often broken off. In *Tubifex* where the cuticle is thinner posterior segments drop off one at a time; in *Limnodrilus* they detach in sections. Meanwhile the whole anterior tip loads heavily with stain. Prior to the sloughing off of segments the purplish stain seems to concentrate heavily along the lateral portions of the segments and then spread elsewhere; otherwise anterior rings stain most prominently at the bristle level and posterior ones in blue bands intersegmentally. Finally the dye concentrates ventrally in each segment. The last parts to stain are the dorsal portions of those segments somewhat behind the head. Rhythmic pulsations of blood vessels and alimentary canal continue regularly until slightly before the deep staining of the approaching death-point is attained.

In disintegration the parts swell greatly and constrict between segments. Usually the posterior half is lost by detachment of rings or groups before the head dies; even in 0.001 per cent m.b. 20 or more anal segments were dropped in 3½ hours. Head and succeeding parts go next, the last surviving segments being a block about one-fourth of the distance back.

The chick embryo. The incubated egg was opened and immersed in a solution of 1 part m.b. powder in 5-10,000 parts of isotonic saline, previously warmed to incubation temperature and held at about that point for the course of the experiment.

The blastodisc, germ wall, and actively growing vascular area stain before the yolk or vegetal pole. The regions most sensitive to coloration are the formative regions: the edges of the uprising medullary folds in the anterior parts, and the sides of the neural groove posterior to the head fold, fading out more posteriorly until the level of forming somites is reached and back toward the anterior end of the primitive streak, where deeper stain is again met with.

On 3-somite chicks the medullary folds and head ectoderm are the most conspicuously stained portions anteriorly, and the end of the folds posteriorly, where some active growth and proliferation is taking place.

Five to seven-somite chicks stain deepest on the head, the intensity thence grading down in the region of formed somites and then gradually rising again in the extending medullary plate and folds. It certainly seems to be clearly possible to distinguish here a descending primary gradient (of differentiated structure) and a posterior ascending (growth) gradient, resembling generally that in annelids. At least the ectoderm of a 10 to 12-somite chick becomes a deep opaque blue-black in the head, which fades out to the end of the region of the closed medullary tube, whence it again deepens toward the posterior ends of the medullary folds. The trough of the medullary groove is comparatively lightly stained. The bulk of the stain accumulates at first only in the ectodermal layer, for this may be peeled off leaving the interior practically uncolored. The mesodermal somite regions, however, evidently show a somewhat similar gradation of superficial staining later, lowering from the head through the formed somites and rising sharply toward the posterior end of the region of developing somites and anterior to the unsegmented plate.

DISCUSSION

In at least three ways the intravital dyes might be expected to contribute to a solution of the problems of differential susceptibility and to a closer study of the real nature of a "dominant region" or active state. (1) The movements of their conspicuously colored particles may be followed more readily than those of most agents, their points and rates of penetration and sites and manner of storage noted, and the facts so

obtained correlated with the results obtained by the susceptibility method with the dyes and with other agents and conditions. (2) Alterations in penetration and storage may be studied: *a*, during different functional states; and *b*, with the dyes in media containing dissolved salts, acids and bases. (3) Use may be made tentatively of the physicochemical properties of the dyes and of protoplasm, as far as known, for analysis of the process of staining and of the manner of production of dye effects upon the organism. This applies to dyes, toxic and relatively non-toxic, vital staining and non-staining, as well to those undergoing some definite change (oxidation, reduction, or a change in color indicating reaction, or a change of state) as to those which apparently resist such changes. Obviously this work makes use of by no means all the possibilities of the method.

1. The susceptibility gradient with dyes. The general significance of the differential susceptibility existing along the structural axis of axiate organisms has been often and fully discussed (2),(11). In the light of the data derived largely from these previous studies with KNC, lack of oxygen, and narcotics, and from the quantitative determination of gas intake and output, there seems to be full warrant for the belief that, under conditions where death occurs at all rapidly, the relative susceptibility indicates more or less delicately a gradient in some slight differences in metabolic activity, associated in all probability with oxidations.

As described in the details of this paper, the numerous basic and rarer acid dyes which are toxic enough in nearly neutral solution to be distinctly lethal, produce disintegration beginning in cephalic parts and other sites of known markedly high metabolic rate—this disintegration agreeing, so far as the facts have been determined, in fundamentals with that for cyanides, etc., in the same forms. The chief divergence in results occurs in the lack of delicacy of the dyes in distinguishing the small differences detected by KNC. Even such dyes as m.b., which undergo oxidation and reduction changes, failed in the same way and were disappointing, inasmuch as at the beginning of the work it was thought likely *a priori* that they might give positive results. Helpful facts should certainly be obtained from the use of methylene blue and its leucobase in conditions where oxygen can be supplied or withdrawn at will.

The nature of the difference in susceptibility to dyes as compared with that to KNC, etc. is interesting and perhaps significant, particularly if compared also with susceptibility to acids and bases (4). It may be

fairly stated that in this matter and in general physiological effects basic dyes behave much like acids (HCl or acetic), and acid dyes much like alkalies (NaOH). Thus both basic dyes and acids produce in flat-worms a most rapid disintegration of the anterior end, often of the ventral surface as compared with the dorsal, sometimes of the midline as compared with more lateral parts; the anterior ends of the second and succeeding zoöids are not singled out for early disintegration; no matter how low the concentration and how slow the killing, disintegration, when it does occur progresses posteriorly; and finally, in any quickly lethal concentration the young and old individuals are nearly equally tolerant, the young being but slightly, if at all, less resistant than the old. Acid dyes, conversely, resemble alkalies in their effects, though seldom sufficiently toxic to be readily lethal except in solutions made considerably acid. Thus alizarin blue S causes most rapid disintegration of the anterior end, dorsal surface and lateral margins, and often of the anterior ends of the second and third zoöids; and young individuals are plainly much less tolerant than older ones; and in certain cases indirect susceptibility was observed.

These similarities of action had not been anticipated, in fact were discovered entirely empirically, but might logically have been expected from the likeness of the dissociation of these agents. The neutral salts of basic dyes hydrolyze freely in neutral or alkaline solution, and dissociate into a large colored basic ion, the cation, which moves toward a cathode, and the anion radical, e.g.,Cl′, of a strong acid. Evidently *the large color ion, like the mobile H ion, predominates powerfully in effect over the anion.* On the other hand, acid dyes dissociate, if at all, into a large colored anion resembling hydroxyl ion in effects, and a less potent metallic ion like Na^+.

The influence of impurities, such as heavy metals, in these dyes deserves treatment, of course, but these did not seem to interfere with the action above described, which I interpreted as due to the dye itself, since all samples of numerous dyes from varied sources gave like effects. If acids are added to acid dyes or alkalies to basic dyes to increase their action the results are naturally neutralized and confusing.

2. Decolorization gradient. That a gradient of decolorization by extrusion or escape of dye and dye particles should be found in some forms, as many flatworms, is not really a new observation. Such loss of stain, like the loss of natural pigments many times reported occurs commonly when death is imminent, and may be taken to indicate that resistance to the passage of diffusing substance has disappeared, and

conditions of normal semi-permeability destroyed, or perhaps, in this case, rather that retaining surface layers have been broken and loosened by beginning disintegration, and allow escape of discrete particles, especially if these are under pressure from adjacent swollen tissue or from a cover slip. Recovery is, however, often still possible after a certain amount of this loss has taken place.

3. The staining gradient. Although a sort of coloration gradient had been reported by Child with potassium permanganate (12) and with indophenol (3), the field of true axial protoplasmic vital staining with dyes has been entered deliberately only in this and associated studies on algae and hydroids (13); hence the facts merit brief discussion here.

Any doubt of the general observation that with easily visible basic dyes, such as methylene blue, toluidin blue and many others, there is produced, as reported for the forms used, a color gradient corresponding approximately to the major susceptibility and metabolic gradients (as determined by HCl rather than by KNC), may be removed by a few simple but convincing experiments such as above outlined, with organisms consisting either of one zoöid or of two or more morphologically distinct zoöids. Generally speaking, the order of staining of parts corresponds to the order of their susceptibility to acids and to the basic dyes themselves. Slight differences of susceptibility as shown by cyanides are not usually indicated either by susceptibility or by staining with these dyes, but it is safe to state that, knowing the order of staining with basic dyes one can quite reasonably predict the general course of disintegration with most lethal agents, or knowing the relative susceptibility of parts, their relative staining times may be foretold.

Concerning the correct interpretation of these findings there may well be some diversity of opinion. Where so little is really known one may not safely become assertive or dogmatic. But I shall point out a few indications of the rôle of some physico-chemical factors underlying, conditioning, or associated with metabolic gradients, to this end invoking the aid of current doctrines. To attempt to apply the classic as well as more recent theories of vital staining, both "physical" and "chemical," may help to recognize, eliminate or evaluate the factors here concerned.

First of all, one may be assured that there is no simple gross anatomical explanation to account for the axial ingress of the stain. The mouth is seldom situated at the cephalic end of the staining gradient, and is often well back, e.g., midway in flatworms, and at the base of

the tentacles in hydra. The rôle of the alimentary canal as a path of staining is easily recognized, as in the cases of *Hydra* or *Aelosoma*. The first parts to dye are usually particles or globules in or subjacent to the epithelial surface and stain passes in through the surface not by any large aperture. Nor is there staining of some special tissue alone, though ciliated and sensory surfaces and nervous elements are oftenest first conspicuous. The gradient is easy and continuous, but need not be always antero-posterior, as differentiated structure would commonly be; in higher annelids the dye enters earliest at the ends, and later in the middle regions. Much the same limitations apply to a "density" factor. If metabolic activity leaves an accumulating residue of organized reserve or inactive stabilizing substance, then as differentiation proceeds this material might be laid down gradientwise and be stained accordingly; but a newly regenerated head, highly transparent, nongranular, and relatively undifferentiated accumulates stain earlier than adjacent posterior levels, and the staining particles appear identical throughout the axis.

In view of Ehrlich's demonstration (7) of the relation existing between staining capacity with methylene blue and the rate of oxidation in different tissues, the assumption was made *a priori*, that stain might enter equally all along the chief axis but become invisible in certain parts through transformation into colorless base by marked local reducing action. That such is not the case here is shown by tests with strong oxidizing agents, as H_2O_2, which fail to reveal, i.e. make blue, any such invisible dye base. On the contrary, there is every reason to believe that a greater power of reduction in deoxygenated water must be possessed by those most active parts, first staining in aerated water: and it seems likely that in deficiency of oxygen supply reducing power is a criterion of vital activity, while in abundance of oxygen staining with methylene blue is such a criterion. Certainly in well oxygenated media any local reducing action may be ignored entirely, especially since in most of the vital stains the colorless form does not exist.

The gradient may, of course, be "explained" and dismissed as due simply to differences along the axis in *permeability* of the membrane to dye particles. If by this term one implies some kind of ultrafilter in the sense of Ruhland (14), then it may I think be rejected, for the size of particles of basic dyes is increased in the same alkaline solutions that facilitate their penetration and the same is true for acid dyes in acid solutions (6). In any case the mechanism of alteration of permeability itself requires analysis.

For the popular Overton theory (15), maintaining that the entrance dyes, basic or otherwise, into nervous and other tissues is determined by their relatively high solubility in lipoids that collect at surfaces and phase boundaries, there is no support here. The theory obviously cannot hold both for the lipoid-soluble dyes (janus green, dahlia, neutral red, methylene blue, methyl violet) and for the similarly acting lipoid-insoluble dyes (toluidin blue, thionin, methyl green), all of which enter and exhibit strikingly like effects in the cases tested. The intravital stains are mostly basic, and many of them have been designated as "specific" nerve stains, but even for the nerve it is not the myelin sheath which is colored, but the neurofibrillae and Nissl bodies (16). Solubility in lipoids would appear to have no prominent influence in controlling distribution or effects of dyes as here described.

Ehrlich and his students, Fischel (17) and Goldman, contended that vital dyes react chemically with definite specific dye-receptors of large protoplasmic molecules, which receptors might conceivably concentrate in graded amounts along the axis and could thus determine and measure the relative affinities of various parts of the animal for the stain. It is hard to refute this view but many facts stand against it as stated in its original form: first, nearly all or all levels parts and tissues of the organism stain *finally* to approximately the same depth; it is merely a difference of time required to bring in the stain and make it visible, not of stainability or of amount of stain taken up ultimately. Second, the marked non-specificity of staining with the various basic dyes points to some more fundamental common property conditioning the reception of the stain. Chemically unlike dyes (thiazins, azo-dyes, etc.) (5), (18) often behave alike, and those of closest chemical relationship are as frequently opposing in effect. Further, practically any lethal agent or condition will produce a disintegration gradient of an essentially similar nature, differing only in minor respects-cyanides, narcotics, lack of oxygen, excess CO_2 or other waste products, salts of heavy metals, dyes and indicators, ions of electrolytes, high and low temperatures, and doubtless a great variety of other unfavorable conditions and of agents of no known or obvious chemical similarity or kinship. As to the dyes, the most prominent cleavage among them is not one of specific chemical constitution but that between acid and basic, a matter of reaction and manner of dissociation. Admitting the probability of some specificity in details of working of individual dyes does not warrant speaking of staining as a specific chemical process. There must be some widespread and less specific chemical or physical character responsible for the course of basic vital staining.

In the writer's opinion the staining gradient may well be due to the greater entrance and fixaton of dyes in certain regions corresponding to a graded difference in adsorption or combining capacity, itself based on metabolic activity. This conception is not inconsistent with the belief that continued entrace of the dye depends in large part on its power of combining with or being precipitated of flocculated by certain constituents of the cells, and that its accumulation is possible because more or less insoluble compounds are formed within (19).

Valid evidence as to the mode of fixation of the dye may be obtained from the study of the conditions necessary for staining of textile fibers, proteins, etc., *in vitro* and *post mortem*. Basic dyes, yielding electropositive colored ions, form insoluble colored salts with many "acid" protoplasmic substances containing organic acids combined with strong bases, e.g., mucin, hyaline cartilage, nuclein, amyloid, casein, Nissl bodies, yolk material, soaps, etc., the dyes combining like metals to form a basic-dye-albuminate, etc.; but basic dyes will not combine with the more basic or neutral albumins, globulins, albumoses, histones, etc., except in alkaline solution. These latter substances, however, especially in acid media, combine readily, often with a precipitation, with free inorganic or organic acids and with acid dyes of all kinds, giving an albumin-acid-dye compound (20), (21), (22). Neutral gelatin combines with neither acid nor basic dyes; if made electropositive it stains with and retains acid dyes (acid fuchsin), and if made electro-negative it takes up and retains basic dyes (neutral red) (23). In general, previous adsorption of acid or neutral salt ions tends to discharge and aggregate electro-negative colloids, and accordingly both *in vivo* and *in vitro* decreases basic staining and increases acid staining, while conversely, alkaline solutions favor basic staining and diminish acid staining (5), (24), (25), (27).

These suggestions by way of an electro-chemical view of staining are supported by the facts of vital staining. Methylene blue and neutral red form insoluble dye-tannate compounds with tannic acid of the sap vacuoles of *Spirogyra* (28); neutral red forms a soluble red compound with some organic acid in *Elodea;* while in animal cells, as in Paramecium and in Echinoderm eggs union is made probably with some lecithoprotein (29). From recorded and new data Von Moellendorf (27) concludes that basic dyes react with natural acid colloid (anion) constituents of cell protoplasm in the same manner as they react with acid dye ions either *in vitro* or stored previously as granules *in vivo;* but basic stained protoplasm cannot thus combine later with acid dyes!

Matthews believes that basic dyes stain because they form some insoluble compound (salts, esters, etc.) with the "proteinate," "lecithinate," amino-acid, cholesterol, fatty acid, and other similar ions of protoplasm. The compounds may be regarded as weak surface chemical combinations quite insoluble and inert chemically, usually but not always highly stable, sometimes slightly dissociating and partly reversible.

That animal protoplasm is usually electro-negative is attested by both electrical and physiological observations; under ordinary conditions and in ordinary slightly alkaline media the proteins and lipo-proteins of which it is composed, having low iso-electric points, dissociate with negative charge (5), (30). Electro-negative colloids behave electrically like anions of an acid, and anions combine with or adsorb positively charged metal or basic dye cations, but not acid dye anions, which wash out leaving no stain. Even a prolonged immersion in an acid medium does not commonly, but may sometimes, suffice to bring about acid dye staining. In the exceptional cases where colloids are positively charged, as in hemoglobin of erythrocytes, perhaps in many plant cells, and apparently in some leeches, it is the acid dyes that really stain. In extreme acidosis also tissues may stain vitally with acid dyes (31). From this view the likeness of the effects of basic dyes and acids, both with predominant cations, is to be expected.

If anions or acid radicals are important requisites of basic staining, then there would need be a graduated production of such anions along the axis to account for the staining gradient, as well as a greater abundance of them in certain tissues, as nervous tissue, and in active parts generally. A constant, and in life unfailing, source of these anions may be sought in the katabolic processes, which yield acid products always on the whole preponderating over the ammonia produced. From split products an increased number of molecules results which, like amphoteric substances in the alkaline interior of the cell, dissociate as acids with a maximum number of anions and a certain number of H ions. This would not necessarily result in any actual acid reaction even locally; one can only speculate as to the disposal of the H ions, whether by neutralization, or by rapid escape outward, or by formation of a Helmholtz double layer; rapid liberation of H ions may produce the galvanometric electro-negativity of the active part (32).

It is interesting to note what has been taken as a curious old observation, that only the color ion of a basic dye is adsorbed, while the Cl ion is left in the outer solution (5). Similarly only the H ions of an HCl solution are absorbed, according to Gray (33), who says that the

charge of the Cl′ outside is satisfied by outgoing K ions, which are themselves replaced by H ions. It seems likely that basic dye ions likewise take the place of H ions, providing these are able to make their escape into the outer medium (as in neutral or alkaline media), but if the H ions are unable to escape (as in acid solutions without, or with acid reaction within the cell) they would block the entrance of the dye ions. For there is considerable evidence that if reaction of the cell actually becomes acid it no longer stains with basic dyes (6). In general it is sufficient if one of the results of rapid metabolism is a correlative net increase, momentarily at least, in negatively charged ions, which constitute the basic staining substance or condition. Naturally if the anions are formed by dissociation of amphoteric proteins as acids, their production should be reduced by acidity and facilitated by alkalinity, by abundant oxygen, rapid diffusion of CO_2, etc.

The rate of entrance of a dye may be controlled not alone by capacity to form compounds within, more or less depending on metabolic activity; conceivably a greater water content of active parts may aid in more rapid diffusion, or katabolic acids may lead to a more aggregate or more swollen condition, which may stain more rapidly. And there are doubtless other unanalyzed factors in the graded permeability along the axis; in the next section high permeability to dyes will be seen to be practically always associated with states of stimulation and activity.

I believe the points to be not witnout significance that all of the dyes of whatever chemical consitution and however varied the details of their staining pictures, were always in substantial agreement as regards the parts first stained as well as in the final staining gradient; that the dye first become visible at the same definite loci (e.g., the auricles of *Planaria*), whether staining exactly the same substance or not; that these loci are practically always the loci of greatest general susceptibility; that all of the really successful vital stains were basic, while the acid dyes were as generally signal failures; and finally that this staining is apparently dependent upon metabolism, since previously killed animals show no staining gradient.

4. The essential similarity of protoplasmic condition in the dominant region, a stimulated region or condition, and in a fertilized egg. Of more than ordinary interest are the variations in staining in different physiological states, particularly that of excitation. For instance, recently traumatized regions, stomach ulcers (34), and healing wounds are characterized by a state of enhanced metabolic activity, increased res-

piratory exchanges, by weakening of the surface layers, and accordingly are stained easily and strongly.

There is in the literature considerable direct evidence that capacity for taking and holding basic stains varies to some extent with metabolic and respiratory activity. For gland cells the facts are fairly complete and all in full agreement. Asher and Garmus (35) and Garmus (35) showed by direct continuous observation that gland cells of the nictitating membrane of the living frog take up much more vital dye of all various degrees of solubility in lipoids (m.b., neutral red, rhodamin, bismark brown, toluidin blue, thionin) when stimulated to great functional activity by pilocarpine than when left unstimulated, and more when left unstimulated than when function was depressed by atropine. After injection of pilocarpine the secretory granules, etc., were more quickly colored, and with time their coloration became strongly intensified. After local or general atropinazition coloration began very late and never became more than weak, although the cells were as richly granulated as resting or normally functioning cells. The difference could not have been due, therefore, to a greater secretion, but must have been brought about, according to the authors, to an alteration in the permeability of the membranes. Keleman has since shown (36) that pilocarpine increases respiratory exchanges and CO_2 production as much as 10 per cent and increases CO_2 of both arterial and venous blood, while atropine diminishes these below normal. Pilocarpine also raises the relative galvanometric electronegativity of gland cells stimulated by it.

For nerve tissue a similar condition apparently obtains. Bethe (16) discovered that during and shortly after the passage of a polarizing current through a living nerve the neurofibrillae lose absolutely all power of primary staining with methylene blue or toluidine blue at the anode but increase their normal capacity for the same dyes at the cathode. After recovery is complete the staining polarity disappears. These physiological changes exactly parallel the electrotonus changes: decreased irritability and conduction at the anode (anelectrotonus) and increased irritability at the cathode (catelectrotonus); that is, primary stainability varies directly with irritability and conductivity. Bethe came to believe after some experimentation with alkalies, distilled water, narcotics, etc., that at the athode there is an increased affinity of the neurofibril protoplasm for a "fibril acid substance," which moves toward or otherwise becomes more abundant and firmly attached at the cathode pole and there makes the fibrils more stainable. Frequent stimulation, strychninization, and a relative predominance of

katabolic processes over anabolic gives a cathodal appearance to staining fibrils; prolonged rest or excessive stimulation gives an anodal appearance. In death and deep narcosis no polarity is produced. According to the view here suggested the cathode may be conceived as stimulating the production of metabolic acids and attracting and withdrawing from the fiber positive ions, in whose places may be substituted the basic dye ions. Neurologists have repeatedly found that an increased amount of basic staining occurs, e.g., in Purkinje cells of the cerebellum and other motor cells, after first stimulations, but that with excessive stimulation ending in fatigue and exhaustion the staining substance or condition vanishes and less and less staining occurs. Pollock and Cluney (37), commenting upon the results of intravital staining of brain cells of mammals with various dyes introduced into the blood stream or under the meninges, say that "any procedure which increases metabolic activity of cells insures a greater degree of intravitam staining" or ingestion of trypan blue.

Matzumoto (38) reports that very dilute (0.00001 per cent) neutral red or nile blue sulphate after 10 hours to 4 days stains characteristic granules of corneal epithelium of the frog. He notes a general parallel between activity of cells and presence of stain, the deeper basal swollen and active cells (which are more acid, according to Unna (39)) staining in many granules of all sizes, while there is progressively less staining in more superficial cells, and none at all in the flat polygonal cells of the surface.

There are many points of likeness, also, between the processes of fertilization (initiation of cell division) and of stimulation. Arbacia eggs shortly after either natural or artificial fertilization show a two to three fold output of CO_2 and of heat, a vastly increased O_2 consumption, a diminished electrical resistance (as in working striated muscle (40), (30)), an increased permeability to salts, water, natural pigments, and an enhanced stainability with methylene blue and neutral red (41). The most deeply staining individual eggs divided first.

In the light of new observations and relevant literature basic vital staining of different cells and tissues and of different parts of an axiate organism may be understood tentatively as indicating differences in staining condition more or less paralleling metabolic activity, which produces substances with anion radicals capable of combining with positively charged ions. It is yet impossible to clearly dissociate this combining capacity from accompanying and secondary (?) differences in water content, ion content, and especially in "permeability." It has

become increasingly obvious as this work progressed that a sharp distinction could hardly be drawn between chemical reactivity, combining capacity, and permeability; and indeed a semi-permeable membrane is not necessarily a definite, physically detachable, primarily static and unchangeable structure, but should be and commonly is (5), (30), (42) regarded as an integral part of the protoplasm, undergoing all of the changes characteristic of living substance, and hence showing with it similar modifications by agents in the medium as well as its alterations during functional activity. In fact concentrated and exposed surface protoplasm almost certainly participates in these changes even more quickly and completely than less available, remoter, interior parts, and the preliminary effect of an agent or physical condition (in compounds produced, altered or destroyed, or in aggregation and solution effects, etc.) may well facilitate or retard subsequent action and penetration of the agent, or effectiveness of the physical condition. Thus surface metabolism and condition may control general metabolism.

Semi-permeability is probably maintained by metabolic activity. It is interesting to see that organisms killed by heat or alcohol stain quite heavily in a fraction of the time required by a living individual, and in the dead animal no gradient appears, but intake of stain is quite uniform throughout, special sites of election being to all appearances absent.

As staining and susceptibility follow each other closely in their respective courses, it is readily conceivable and probably true that, though cells apparently uninjured and with unaltered membrane take up the stain, a state of excitation, such as we must suppose exists in a dominant part, amounts substantially to a mild injury; that there are all degrees of injury up to death itself; and that staining, like permeability, increases in general after injury and as death approaches.

A region or state of dominance in an axiate organism is thus characterized by possessing distinct differences in susceptibility, in regenerative capacity (2), in respiratory activity (3), (43), in electrical potential (32), probably in catalase content or activity (44). Evidence of differences in rate of vital staining or in permeability has been presented in this paper. In most of these properties the dominant region resembles any metabolically active or stimulated part. Whether it resembles an actively functioning part in certain further respects is yet to be determined. An examination for high water content may be made by analysis; heat production may be measured in favorable forms by some thermocouple device; and electrical conductivity may be quantitatively determined.

In view of the exact and extended parallels which may be drawn between the physiological state of the dominant region and that in any stimulated region it is helpful to conceive of the dominant region in general as a portion in a condition of more or less permanent tonus or continuous partial stimulation, more highly irritable and more quickly responsive than less active parts, and therefore as always activating or stimulating subordinate levels. It responds to stimulation after a briefer latent period, or period of restitution and recovery. Since its activity is but little arrested after responses it is relatively non-fatiguing (like nerve as compared with muscle), and because of these attributes it possesses to a greater degree than other parts automatism and spontaneity, the capacity of "initiating" impulses.

SUMMARY

These researches were undertaken to determine further the nature, characteristics and mode of action of metabolically active parts or tissues, especially those of the "dominant" (cephalic, apical, anterior, etc.) region, chiefly with the aid of a representative series of stains.

The methods employed were those of direct susceptibility and differential vital staining.

1. Both basic and acid dyes were used, but with few exceptions basic dyes alone were found to be sufficiently penetrating and toxic in nearly neutral solutions to be effective.

2. Neutral salts and H ions in the medium retard visible staining with basic stains; OH ions and higher temperature facilitate it, as do also local injury and local healing.

3. Data are given, especially for methylene blue, demonstrating more or less satisfactorily: a staining gradient, sometimes a decolorization gradient, and always a disintegration gradient in the forms used, including *Paramecium*, *Dileptus gigas*, *Hydra oligactis*, several flatworms and oligochetes; and a staining gradient for the chick embryo. The loci of highest general direct susceptibility to most agents and conditions are first to stain visibly with the dyes, first to decolorize (in case color is lost) as death approaches, and first to disintegrate at death.

4. The disintegration gradient with basic dyes resembles that obtained with acids; disintegration with lethal acid dyes resembles the type obtained with alkalies and KNC (p. 361).

5. Basic dye color ions are positively charged, and evidently are taken up by negatively charged colloids and other anions, which there is

reason to believe are most numerous in metabolically active regions, where acid substances are produced (pp. 378–380).

6. There is much collateral evidence to the effect that a difference in permeability accompanies states and sites of greater metabolic activity; but there appears to be little justification as yet for distinguishing sharply between combining capacity and penetration power, or to attempt to give priority to either.

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